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Signatures for chronic obstructive pulmonary disease (COPD) and asthma: a comparative genetic analysis

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) and asthma are obstructive lung diseases which progress in severity with time. Environmental causes and genetic makeup of individuals play important roles in disease manifestation. The aim of present study was to search for diagnostic/prognostic biomarkers to differentiate COPD and asthma.

Materials and methods: Seven *ADAM33* and two *AQP5* single-nucleotide polymorphisms (SNPs) were genotyped by polymerase chain reaction-restriction fragment length polymorphism method. The association of genotypes, haplotypes and allelic combination of variants in different genes was analyzed in 194 COPD, 150 asthma patients and 220 controls.

Results: The genotype frequencies of SNPs V4(C/G), T1(T/C), S2(G/C) of ADAM33 and AQP5 A/G (rs3736309) were associated with COPD and asthma (P=0.038 to P<0.001), while S1(A/G) and F+1(C/T) were associated with asthma (both P<0.001) and V1(G/T) with 20 COPD (P<0.001). The allele frequencies of V4(C/G) (both P<0.001), V1(G/T) (both P<0.05), S2(G/C) (both P<0.01) and S1(A/G) (both P<0.05) were associated with COPD and asthma, while F+1(C/T) was associated only with asthma (P=0.005). Haplotypes of ADAM33 'GGTGGGT' (P=0.027), 'CGTCGGC' (P<0.001) and AQP5 'GA' and 'AG' (both P<0.001) were significant only in COPD.

Conclusion: *ADAM33* F+1(C/T) variant and allele combination 'GGTGGGTGA' may be specific markers for asthma, while AQP5 'AG' appeared as a haplotype associated only with COPD. These specific genetic biomarkers may be exploited to predict individual predisposition to COPD and asthma.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease which may be preventable, treatable, but not fully reversible [1] while asthma is characterized by reversible airflow obstruction due to bronchoconstriction (airway narrowing), airway wall thickening and increased mucous secretion [2]. COPD ranked third among the top 10 causes of death worldwide, while 339 million people suffered from asthma worldwide with 417,918 deaths in 2016 [3,4]. The primary cause of COPD is long-term exposure to tobacco smoke (cigarette, pipe, cigar, etc.) and other pollutants while the exact cause of asthma is not known but allergy stimulates symptoms of the disease. However, neither all tobacco smokers develop COPD nor all allergies lead to asthma. Therefore, in addition to environmental causes, genetic makeup of individuals also plays an important role in disease.

A disintegrin and metalloproteinase 33 (ADAM33), an important protease, is specifically expressed in basal epithelial cells and vascular endothelium of lung airway epithelia and in smooth muscle cells [5]. It plays a role in proteolytic release of cell surface membrane proteins such as cytokines, growth factors, receptors [6] and cleavage of α2-macroglobulin which has important role in pulmonary defence [7]. ADAM33 normally performs airway remodelling, but in a pathogenic state, altered ADAM33 protein may enhance inflammation, thus, increasing the severity of the disease [8]. Single-nucleotide polymorphisms (SNPs) in *ADAM33* may alter the function of ADAM33 protein. Studies have reported that SNPs in *ADAM33* were associated with an accelerated decline of lung function in COPD as well as asthma patients [9]. The obstruction in COPD is not reversible while in asthma it is, and *ADAM33* is one of the common remodelling genes involved in both diseases.

Aquaporins (AQPs) are a family of water channel proteins discovered in the early 1990s [10] and are found in airways and distal lungs [11]. *AQP5*, located on 12q13, codes a product that is expressed in airway epithelial cells, type I alveolar epithelial cells and submucosal gland acinar cells in lungs [12]. AQP5 performs various functions such as maintaining water permeability of cell membrane, nerve signal transduction, skin flexibility, fat metabolism, membrane permeability

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to gases, cell migration and proliferation [13]. There is an association of decreased expression of human AQP5 with an overproduction of mucus in the airways of subjects and a significant reduction in lung function [14,15]. AQP5 may be involved in fluid secretion in submucosal glands. Its luminal membrane poses a restriction on water movement and the expression of AQP5 may provide a treatment in hyperviscous glands [16]. Zhang et al. [17] suggested that AQP5 dysfunction could play a critical role in pathogenesis of asthma and could serve as a biomarker for its diagnosis. The SNPs in *AQP5* were reported to be associated with lung function decline in COPD in Chinese European and American populations [18,19].

In the present study, the association of seven *ADAM33* and two *AQP5* genetic variants viz., V4(C/G), V1(G/T), T1(T/C), S2(G/C), S1(A/G), Q1(G/A), F + 1(C/T), *AQP5* G/A and *AQP5* A/G, respectively, with COPD and asthma was evaluated. We hypothesize that genetic variants of *ADAM33* and *AQP5* are differentially associated with COPD and asthma. The difference in association may help identify a genetic marker which can be used as diagnostic/prognostic marker and can differentiate between COPD and asthma.

Materials and methods

Subjects with COPD (n = 194) or asthma (n = 150) were enrolled from the outpatient department of Respiratory Medicine at King George's Medical University (KGMU), Lucknow. The work was initiated after approval from the Institutional Ethics Committee (533/Ethics/R.Cell-17 dated 26.05.2017) KGMU, Lucknow, India. Age and sex-matched health controls (n = 220) were included in the study from staff members of both universities.

Inclusion criteria for COPD subjects were post-bronchodilator forced expiratory volume (FEV1)/forced vital capacity (FVC) ≤70%, post-bronchodilator FEV1< 200 ml and ≤12% change in FEV1. For asthma subjects, these were post-bronchodilator FEV1/FVC ≤80%. post-bronchodilator FEV1 ≥ 200 ml and ≥12% change in FEV1, whilst controls had a post-bronchodilator change in per cent FEV1/FVC ≥80%, and no family history of any specific disease/allergy/infection/inflammatory responses including COPD and asthma. Exclusion criteria were acute respiratory distress syndrome, history of poorly controlled associated diseases such as heart disease, thyroid disorders, coagulation and haematological disorders, and unable to sign informed consent forms.

Initially, the minor allele frequency (MAF) for each SNP was obtained by genotyping 100 control samples. MAF values obtained were used to calculate the sample size for genotyping each SNP by QUANTO software (ver. online). The disease prevalence was taken as 6.0, and power of study was 80%.

Anthropometrc details such as age (years), body mass index (BMI) and smoking status were recorded. The clinical details included the pulmonary function test (PFT) parameters both pre and post administration of bronchodilators, measuring the lung capacities of COPD, asthma and controls. The parameters of the PFT include the total volume of air exhaled from lungs (the FVC) and the percentage of FVC forced out in first second (FEV1).

A 2 ml peripheral blood sample was collected in EDTA vials from each subject. Genomic DNA was extracted using salting out method [20] with slight modifications [21] from peripheral blood mononuclear cells.

Genotyping of seven *ADAM33* and two *AQP5* SNPs was performed by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR–RFLP). The primers were designed by Primer 3.0 online software, and restriction enzymes were identified using NEB cutter (ver online) (Table 1). The constituents of PCR for 15 µl reaction mixture were 100 ng of template DNA, buffer (100 mM Tris, pH 9.0; 500 mM KCl; 15 mM $MgCl_2$; 0.1% gelatin), 200 µM dNTP, 10 pmol of each primer and 1.0 unit Taq DNA polymerase. PCR products were digested by respective restriction enzymes (New England Biolabs, USA) (Table 1). The resulting digested fragments representing genotypes were electrophoresed on 2.5% agarose gels.

Allele and genotype frequencies of controls and cases were compared using chi-square χ^2 test and Fisher's exact t-test by SPSS software (ver. 21.0). All P values were two-sided, and differences were considered statistically significant for $P < 0.05$. Odds ratio (OR) at 95% confidence intervals (CIs) was determined to describe the strength of association in a logistic regression model. Anthropometric and clinical parameters of controls and cases were non-normally distributed and so compared using Kruskal–Wallis test. Significant Kruskal–Wallis test results were then checked by Mann–Whitney U test for comparing differences among groups. The haplotype and gene–gene interaction analysis of *ADAM33* and *AQP5* variants was performed using the SHEsisPlus software (ver. online) [22].

Results

There were no significant differences between age among the three study groups, but BMI, sex, smoking and all PFTs were significantly different (Table 2). The genotype and allele frequencies were analysed in COPD and asthma cases and compared with controls. The genotype frequencies (Table 3) of SNPs V4 (rs2787094), T1 (rs2280091), S2 (rs528557) of *ADAM33* and *AQP5* rs3736309 were associated with both COPD and asthma. The SNP F + 1(rs511898) showed significant association only with asthma while V1 (rs543749) was associated with COPD. The haplotype analysis of

Table 1. Primers, restriction enzymes used in PCR-RFLP and resulting fragments depicting different genotypes of *ADAM33* and *AQP5* SNPs.

S. no.	rs no. (SNP)	Primer (5′–3′)	Restriction enzymes	Fragment size (bp)
		ADAM33		
1.	V4 (C/G) (rs2787094)	F- GGAGAAGACAGGGTGGGAAG R- CTGGGAGTCTGGTGTGTCTC	*Bpm*I	CC: 140, 58 CG: 198, 140, 58 GG: 198
2.	V1(G/T) (rs543749)	F- CCTGCAGTTCAAGTTCCTGG R-TGGATCACTGGTCCTCACTG	*Bsu*361	TT: 140, 73 GT: 213, 140, 73 GG: 213
3.	T1 (T/C) (rs2280091)	F- CATGAGCCCTTCCCTTCTCC R- GGAGGCAATAACCCACTCAG	*Nco*I	TT: 114, 95 TC: 209, 114, 95 CC: 209
4.	S2 (G/C) (rs528557)	F- AGAGCTCTGAGGAGGGGAAC R- GCAGACCATGACACCTTCCT	*Bsa*H1	GG: 211 GC: 211, 147, 64 CC: 147, 64
5.	S1(A/G) (rs3918396)	F- GGCTGAAAGTATGCCAGTGG R- CTGGGAGTCGGTAGCAACA	*Bts*CI	GA: 184, 125, 59 AA:125, 59 GG: 184
6.	Q1 (G/A) (rs612709)	F- AGTCAGGCAGCGCTGAAG R- GACTTGTCGGGGAGCCTT	*Bse*RI	AG: 238,135,103 AA: 135, 103 GG: 238
7.	F + 1(C/T) (rs511898)	F-CTGCCACAATGTACAGTTCCAGGT R-GAGTGGGAATGCTGTATCTATAGC	*Bsm*B1	TT: 253 CT: 253, 215, 38 CC: 215, 38
		AQP5		
8.	G/A (rs3782322)	F- AGGGTCAGGATGAAAGGAGC R- ATGATCACTCCAAGGCTCCC	*Bso*BI	GG: 169, 47 AG: 216, 169, 47 AA: 216
9.	A/G (rs3736309)	F- CTGGGTGAGTCTGTCCCTTC R- CATACAAAGCCACCTCTGCC	*Xba*I	GG: 97, 97 AG: 194, 97, 97 AA: 194

SNP = single-nucleotide polymorphism; bp = base pair.

Table 2. Details of anthropometric and clinical characteristics in controls, COPD and asthma cases.

Characteristics	Controls (n = 220)	COPD (n = 194)	Asthma (n = 150)
Sex (M:F)	160:60	172:22	101:49
Smoker (yes/no)	113/107	173/21	15/135
Age (years)	46.6 [10.1]	45.9 [7.5]	45.3 [11.7]
BMI (kg/m^2)	22.8 [4.6]	19.6 [3.4]	22.8 [3.2]
Pre FVC (l)	2.9 [0.8]	2.1 [0.7]	2.3 [0.8]
Post FVC (l)	2.9 [0.8]	2.2 [0.7]	2.4 [0.9]
Pre FEV1 (l)	2.4 [0.7]	1.2 [0.5]	1.5 [0.5]
Post FEV1(l)	2.5 [0.7]	1.3 [0.5]	1.8 [0.4]
Post FEV1/FVC (%)	95.1 [9.9]	56.9 [8.9]	71.4 [10.6]

Data mean [SD] or number of subjects and analysed by Kruskal–Wallis test or chi-squared. All differences $P < 0.001$ except age $P = 0.448$. M = male; F = female; pre FVC = force vital capacity before bronchodilator; post FVC = forced vital capacity after bronchodilator; pre FEV1 = forced expiratory volume in 1 s before bronchodilator; post FEV1 = forced expiratory volume in 1 s after bronchodilator.

seven SNPs in *ADAM33* viz., V4(C/G), V1 (G/T), T1(T/C), S2(G/C), S1(A/G), Q1(G/A) and F + 1(C/T) and two *AQP5* promoter G/A and intron3 A/G in COPD and asthma cases are shown in Table 4. *ADAM33* haplotype 'GGTGGGT' and 'CGTCGGC' were significant only in COPD. The result showed four different combinations of *AQP5* promoter G/A and intron3 A/G. *AQP5* haplotype 'GG' was associated in both COPD and asthma while haplotype 'GA' and 'AG' were associated in COPD only (Table 4) when compared to controls. The linkage disequilibrium analysis in *ADAM33* in COPD was 54% while in asthma it was 49%. In asthma cases, *AQP5* showed 41% linkage disequilibrium, 39% in COPD.

When the interaction of seven *ADAM33* and two *AQP5* variants was analysed together in controls, COPD and asthma cases, three combinations – 'GGTGGGTGA', 'CGTCGGCGA' and 'CGTCGGCAA' – showed significant protection against COPD, while the combination 'CTTGGGCGG' was higher in frequency in cases, increasing the link with COPD almost 2.6-fold (Table 5). We did not find any combination to be associated with asthma, but combination 'GGTGGGTGA' was present in higher frequency in controls showing protection against asthma risk (Table 5).

Discussion

Alterations in *ADAM33* have been implicated in airway remodelling [23]. We hypothesized a unique genetic signature for COPD and asthma so as to differentiate between the two obstructive diseases on the basis of genetic markers.

We observed that the genotype and allele frequencies of *ADAM33* variants, V4C/G and S2G/C, were associated with both COPD and asthma. The association results of this study are similar to those reported in different populations in COPD and asthma [9,24–26]. The genotype frequency of T1T/C showed a significant association with COPD but weak association with asthma. However, the distribution of alleles was not significantly different in controls and cases. Unlike the present study, most studies have not found any association of S1 with asthma in Thai, white and black Americans, Chinese Han, Germans, Australians, Hispanic trios and populations from Cartagena, Columbia [24, 27–30]. The allele frequency

Table 3. Analysis of genotype frequencies in COPD (n = 194) and asthma (n = 150) cases compared to healthy controls (n = 220).

Gene/ variant	Genotype	Controls (%)	COPD (%)	*P*	Asthma (%)	*P*
ADAM33						
V4 (C/G) (rs2787094)	GG	60 (27.3)	90 (46.4)	<0.001	93 (62.0)	<0.001
	GC	104 (47.3)	76 (39.2)		46 (30.6)	
	CC	56 (25.4)	28 (14.4)		11 (7.4)	
V1(G/T) (rs543749)	GG	120 (56.0)	102 (52.6)	<0.001	70 (46.6)	0.071
	GT	80 (36.0)	52 (26.8)		55 (36.7)	
	TT	20 (8.0)	40 (20.6)		25 (16.7)	
T1 (T/C) (rs2280091)	TT	103 (48.8)	119 (61.3)	0.007	82 (54.6)	0.038
	TC	98 (42.8)	45 (23.2)		48 (32.0)	
	CC	19 (8.4)	30 (15.5)		20 (13.4)	
S2 (G/C) (rs528557)	GG	135 (61.4)	94 (48.5)	0.025	67 (44.7)	<0.001
	GC	68 (30.9)	89 (45.8)		53 (35.3)	
	CC	17 (7.7)	11 (5.7)		30 (20.0)	
S1(A/G) (rs3918396)	GG	159 (72.3)	123 (63.4)	0.149	74 (49.4)	<0.001
	GA	39 (17.7)	47 (24.2)		53 (35.3)	
	AA	22 (10.0)	24 (12.4)		23 (15.3)	
Q1 (G/A) (rs612709)	GG	143 (65.0)	118 (60.8)	0.507	88 (58.7)	0.453
	GA	57 (25.9)	52 (26.8)		47 (31.3)	
	AA	20 (9.1)	24 (12.4)		15 (10.0)	
F + 1 (C/T) (rs511898)	CC	76 (34.6)	63 (32.5)	0.790	44 (29.3)	<0.001
	CT	120 (54.5)	106 (54.6)		66 (44.0)	
	TT	24 (10.9)	25(12.9)		40 (26.7)	
AQP5						
G/A (rs3782322)	GG	111 (50.4)	79 (40.7)	0.131	84 (56.0)	0.377
	GA	89 (40.9)	96 (49.5)		50 (33.3)	
	AA	20 (9.1)	19 (9.8)		16 (10.7)	
A/G (rs3736309)	AA	102 (46.4)	126 (64.9)	<0.001	78 (52.0)	0.002
	AG	75 (34.1)	58 (29.9)		62 (41.3)	
	GG	43 (19.5)	10 (5.2)		10 (6.7)	

<0.05 = significant.

of S1A/G was significantly associated with COPD, several populations. Hispanic, Northeastern Chinese, Egyptian, Mongolians of China and South Indians showed a significant association with COPD risk as in the present study [9,31–34]. This differential association of alleles of S1A/G may have a role in tissue remodelling by ADAM33 in asthma and hence the reversibility Q1(G/A) did not show any association with COPD or asthma, confirming other data [28]. However, several studies have showed a significant association in different populations, potentially due to ethnic/racial variations [9,29,31–33,35].

The genotype frequency of *ADAM33* F+1C/T did not show any association with COPD while the association with asthma was highly significant. However, allele 'T' was more frequent in COPD (40.2%) and asthma (48.7%) and appeared to increase the risk by ≥1.5-fold. There are varied reports showing association of this SNP. In a German population, there was no association with COPD [36], while a significant association was observed

Table 4. Association of *ADAM33* and *AQP5* haplotypes with controls (n = 220), COPD (n = 194) and asthma cases (n = 150).

***ADAM33* V4(C/G), V1(G/T), T1(T/C), S2(G/C), S1(A/G), Q1(G/A), F + 1(C/T)**				
Haplotype	Controls *N* (%)	COPD *N* (%)	OR (95% CI)	*P*
CTTGGGT	27 (5.4)	21 (5.2)	0.97 (0.54–1.74)	0.92
CGCGGGT	16 (3.2)	15 (3.7)	1.18 (0.57–2.41)	0.653
GGTGGGT	55 (11.0)	27 (6.7)	0.59 (0.36–0.95)	0.027
CGTCGGC	75 (15.0)	32 (8.0)	0.49 (0.32–0.76)	0.001
CGTGGGC	35 (7.0)	36 (9.0)	1.33 (0.81–2.13)	0.268
	Controls *N* (%)	Asthma *N* (%)		
CGTGGGC	51 (11.5)	32 (10.6)	0.91 (0.57–1.46)	0.695
CTTGGGT	15 (3.4)	10 (3.3)	0.98 (0.43–2.21)	0.955
CGCGGGT	18 (4.0)	17 (5.6)	1.41(0.71–2.78)	0.321
***AQP5* (promoter G/A, intron3 A/G)**				
	Controls *n* (%)	COPD *n* (%)		
GA	230 (52.2)	138 (35.5)	0.50 (0.38–0.67)	<0.001
GG	76 (17.2)	128 (32.9)	2.36 (1.70–3.26)	<0.001
AA	119 (27.0)	96 (24.7)	0.89 (0.65–1.21)	0.45
AG	15 (3.4)	26 (6.7)	2.03 (1.06–3.90)	0.029
	Controls *N* (%)	Asthma *N* (%)		
GA	230 (52.2)	149 (49.6)	0.9 (0.67–1.21)	0.486
GG	76 (17.2)	69 (23.0)	1.43 (0.99–2.06)	0.053
AA	119 (27.0)	67 (22.3)	0.78 (0.55–1.09)	0.146
AG	15 (3.4)	15 (5.0)	1.49 (0.72–3.10)	0.281

Controls are the reference group. CI = confidence interval; OR = odds ratio.

Table 5. Allele combinations of gene *ADAM33* (V4C/G, V1G/T, T1T/C, S2G/C, S1A/G, Q1A/G and F + 1 C/T) and *AQP5* (promoter G/A, intron3 A/G) in controls (n = 220), COPD (n = 194) and asthma cases (n = 150).

			P	OR (95% CI)
ADAM33 haplotype	Controls *n* (%)	COPD *n* (%)		
CTTGGGCGG	9 (1.8)	18 (4.5)	0.018	2.57 (1.14–5.79)
GGTGGGTGA	25 (5.0)	5 (1.2)	0.001	0.24 (0.09–0.63)
CGTCGGCGA	39 (7.8)	12 (3.0)	0.001	0.37 (0.19–0.71)
CGTCGGCAA	31 (6.2)	10 (2.5)	0.008	0.39 (0.19–0.80)
CGTGGGCGA	22 (4.4)	13 (3.2)	0.375	0.73 (0.36–1.47)
CTTGGATGA	23 (4.6)	17 (4.2)	0.8	0.92 (0.48–1.75)
AQP5 haplotype	Controls *n* (%)	Asthma *n* (%)		
GGTGGGTGA	46 (9.2)	9 (3.0)	<0.001	0.31 (0.15–0.63)
CGTGGGCAA	16 (3.2)	11 (3.6)	0.723	1.15 (0.53–2.52)
CTTGGATGA	16 (3.2)	10 (3.3)	0.917	1.04 (0.47–2.33)
CGCCGGCGA	21 (4.2)	11 (3.6)	0.709	0.87 (0.41–1.83)

Controls are the reference group. CI = confidence interval; OR = odds ratio.

Table 6. Analysis of allele frequencies in COPD (total alleles = 388) and asthma (total alleles = 300) cases compared to controls (healthy subjects) (total alleles = 440).

			COPD			Asthma		
	Allele	Controls *n* (%)	COPD *n* (%)	*P*	OR (95% CI)	Asthma *n* (%)	*P*	OR (95% CI)
***ADAM33*variants**								
V4(C/G) (rs2787094)	G	224 (50.9)	256 (65.9)	<0.001	0.54 (0.40–0.71)	232 (77.3)	<0.001	0.30 (0.22–0.42)
	C	216 (49.1)	132 (34.1)			68 (22.7)		
V1(G/T) (rs543749)	G	320 (72.7)	256 (65.9)	0.036	1.38 (1.02–1.85)	195 (65.0)	0.025	1.44 (1.05–1.97)
	T	120 (27.2)	132 (34.1)			105 (35.0)		
T1(T/C) (rs2280091)	T	304 (69.1)	283 (72.9)	0.224	0.83 (0.61–1.12)	212 (70.6)	0.647	0.93 (0.67–1.27)
	C	136 (30.1)	105 (27.1)			88 (29.4)		
S2(G/C) (rs528557)	G	338 (76.8)	277 (71.4)	0.006	1.58 (1.14–2.17)	187 (62.3)	<0.001	2.38 (1.7–3.31)
	C	86 (23.2)	111 (28.6)			113 (37.7)		
S1(A/G) (rs3918396)	G	357 (81.1)	293 (75.5)	0.05	1.39 (1.00–1.94)	201 (67.0)	<0.001	2.12 (1.51–2.97)
	A	83 (18.9)	95 (24.5)			99 (33.0)		
Q1(G/A) (rs612709)	G	343 (77.9)	288 (74.2)	0.209	1.23 (0.89–1.69)	223 (74.3)	0.254	1.22 (0.87–1.72)
	A	97 (22.1)	100 (25.8)			77 (05.7)		
F + 1(C/T)	C	272 (61.8)	232 (59.8)	0.551	1.09	154 (51.3)	0.005	1.54
(rs511898)	T	168 (38.2)	156 (40.2)		(0.82–1.44)	146 (48.7)		(1.14–2.07)
AQP5 variants								
G/A (rs3782322)	G	311 (70.7)	254 (65.5)	0.108	1.27	218 (72.7)	0.557	0.91
	A	129 (29.3)	134 (34.5)		(0.95–1.71)	82 (27.3)		(0.65–1.26)
A/G	A	279 (63.4)	310 (79.9)	<0.001	0.44	218 (72.7)	0.009	0.65
(rs3736309)	G	161 (36.6)	78 (20.1)		(0.32–0.59)	82 (27.3)		(0.47–0.89)

<0.05 = significant. CI = confidence interval; OR = odds ratio.

in Hispanics and Chinese Hans [9,31,33,35]. In asthma, our results are in agreement with previously reported studies [26,37]. A meta-analysis of 29 studies reported an overall significant association of F + 1 with asthma [38]. On the contrary, Chinese, German and Australian populations did not show any significant association with asthma [28,29].

AQP5 has potential role in airflow limitation and mucous overproduction in COPD, which is evident by its altered expression [14]. Direct evidence of correlation between asthma and AQP5 was provided, and it was suggested that AQP5 levels could be used as a moderate biomarker for the diagnosis of asthma in a clinical setting [17]. In the present study, *AQP5* intron3A/G (rs3736309) variant was different in allele distribution among controls, COPD and asthma cases. The frequency of 'AA' genotype and 'A' allele was high in COPD and asthma cases when compared to controls (Table 3 and Table 6). This can be attributed to the involvement of 'A' in disease risk. The frequency of minor allele 'G' was higher in controls than COPD and asthma cases, hinting at a role in protection from the disease. Our results are in agreement with the studies conducted in Chinese, Europeans and Americans, which reported that allele 'G', although lower in prevalence, is protective as it is associated with a significant attenuation in lung function decline among smokers [18,19]. *AQP5* promoter G/A (rs3782322) was not linked to COPD or asthma. No study was found to be disconcordant with ours. To date, we have not found any reports investigating the association of *AQP5* promoter G/A (rs3782322) and *AQP5* intron3 A/G (rs3736309) SNPs with asthma.

In a genome-wide association study, strong linkage was found in SNPs of *ADAM33* [39,40]. In the present study, haplotype analysis of all seven SNPs in *ADAM33* showed a strong linkage disequilibrium in COPD (D' = 0.47) and asthma (D' = 0.41). Therefore, *ADAM33* haplotypes have a high probability of being inherited together.

Out of 102 haplotypes, 'GGTGGGT' and 'CGTCGGC' showed significant association, and none appeared to increase the COPD risk, but instead they were protective against COPD, although with a low OR. None of the

ADAM33 haplotype were associated with asthma. 'GG' of *AQP5* is common risk haplotype for COPD and asthma. *AQP5* haplotype 'GA' was protective against the COPD while 'AG' appeared to increase the COPD risk, although the number of individuals having this genotype is low. In the gene–gene interaction of *ADAM33* and *AQP5*, we found one combination 'GGTGGGTGA' to be protective against asthma. In case of COPD allele combinations, 'GGTGGGTGA', 'CGTCGGCAA' and 'CGTCGGCGA' were higher in controls, hence showing protection against COPD. Combination 'CTTGGGCGG' was higher in frequency in COPD cases, increasing the risk by 2.6-fold.

The association of *ADAM33* F + 1 C/T was significant (both genotypic and allelic) in asthma cases alone and, therefore, can be exploited as a genetic marker to differentiate asthma cases from COPD. Another interesting observation was that 'AG' appeared as a unique risk haplotype associated only with COPD and not with asthma. The haplotype 'AG' may be used to predict the predisposition of COPD in healthy individuals. Combination 'CTTGGGCGG' was associated uniquely with COPD and has high prevalence and so may be used as a genetic signature. Further, functional analysis of these polymorphisms will give us a clear picture of their role in the specific diseases.

Our data represent a significant advance in biomedical science because it shows that combinations of SNPs in *ADAM33* and *AQP5* can be diagnostic markers for COPD and asthma.

Summary table

What is known about this subject?

- COPD and asthma are obstructive diseases with similar symptoms, i.e. breathlessness; however, there is a basic difference of reversibility.
- *ADAM33* and *AQP5* are important candidate genes associated with these diseases.

What this study adds?

- Comparative analysis of gene variants in *ADAM33* and *AQP5* was found to be differentially associated with COPD and asthma.
- *AQP5* haplotype 'AG' and combination of nine variants 'CTTGGGCGG' in gene–gene interaction were associated with COPD alone.
  ADAM33 variant F+1C/T appeared as a unique genetic signature associated with asthma.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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