

GENOTYPES

ADAM33 Gene Polymorphisms Associate with Asthma Susceptibility and Severity in East China Han Population

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Objective. Multiple genetic and environmental factors impact the pathogenesis of asthma. *ADAM33* (a disintegrin and metalloproteinase domain 33) represents a novel susceptibility gene for asthma in several diverse populations. The aim of this study was to determine whether single nucleotide polymorphisms (SNPs) of the *ADAM33* gene associate with asthma susceptibility and severity in the Chinese Han population. **Methods.** A total of 224 subjects were enrolled, including 74 normal controls and 150 asthmatic patients. The asthmatic enrollees were further categorized into high- or low-severity groups according to the percentage of forced expiratory volume in 1 second of predicted value, symptoms, nighttime awakening, requirement for short-acting β_2 -agonist, and interference with normal activities. Six SNPs (F + 1, ST + 4, S1, S2, T1, and T2) in *ADAM33* were genotyped using the polymerase chain restriction fragment length polymorphism method. **Results.** Three SNPs (F + 1, T1, and T2) of *ADAM33* were found to have significant associations with asthma in the study population ($p = .0058-.0067$). The allele frequencies of two SNPs (F + 1, T1) in both the low- and high-severity groups were significantly different from the allele frequency in the control group. The allelic frequency of the T2 SNP was significantly different from that of the control group only in the high-severity group ($p = .0081$). Haplotype analysis demonstrated that the frequency of 7575G, 12433T, and 12462C (GTC haplotype) is higher in healthy controls than amongst asthma patients (78.4% vs. 61.8%, $p = .0004$). **Conclusions.** Polymorphisms of the *ADAM33* gene associate with asthma susceptibility in the east China Han population, and the genetic association is stronger in high-severity asthmatics.

Keywords *ADAM33*, asthma, single nucleotide polymorphism, susceptibility gene

INTRODUCTION

Asthma is a common respiratory disease in which chronic inflammation leads to an airflow obstruction and bronchial hyperresponsiveness (BHR). While it is clear that environmental factors contribute to the pathogenesis of asthma, multiple genes also participate in the susceptibility and progression of this disease (1). Through positional cloning in a genetic linkage and association study of asthma and BHR, *ADAM33* (a disintegrin and metalloproteinase domain 33), located on the short arm of chromosome 20, has recently been identified as an asthma susceptibility gene (2).

ADAM33 is a highly polymorphic locus containing more than 70 single nucleotide polymorphisms (SNPs) (2, 3). It encodes a (single or multiple) pass integral membrane glycoprotein, and sequence analysis predicts several domains: catalytic, metalloprotease, disintegrin (binds integrins), and cysteine-rich/epidermal growth factor (cell–cell contact). Based on the character of these domains, it is thought that the protein product of *ADAM33* should participate in cellular adhesion and fusion, proteolysis, ligand activation, and signaling. *ADAM33* is preferentially expressed in stromal cells, such as fibroblasts and smooth muscle cells, rather than in epithelial cells and T cells or inflammatory

leukocytes (2, 4). Recent studies found that increased levels of *ADAM33* mRNA and protein expression were detected in bronchioalveolar lavage fluid or bronchial biopsies from patients with asthma (5–7). Our previous study also demonstrates that *ADAM33* mRNA expression is increased in the lungs of a murine model of allergen-induced chronic airway inflammation (8). These results suggest that *ADAM33* might be involved in BHR and small-airway remodeling.

ADAM33 has been extensively characterized at molecular and structure levels since its discovery in 2002. However, its biological role and relationship to asthma have remained elusive. Several studies have attempted to characterize the contribution of *ADAM33* to asthma susceptibility and related phenotypes. One investigator found that at least one SNP of *ADAM33* was associated with asthma in African Americans, US Hispanics, US whites, and Dutch whites in the United States and the United Kingdom (9). Other studies reported that *ADAM33* associates with asthma in German (10), Japanese (11), and Korean populations (12). One variant in *ADAM33* was found to be important not only in the development of asthma, but also in the progression of the disease in Dutch Caucasian patients (13). However, *ADAM33* does not associate with asthma in all populations. For instance, it does not associate with asthma in Latino Americans (Puerto Rican or Mexican) (14). In addition, *ADAM33* polymorphisms did not associate with asthma or related phenotypes in a large North American pediatric population (white, Hispanic, and African American) (15).

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These studies suggest that the contribution of *ADAM33* to asthma may be race specific. In China, association studies of *ADAM33* polymorphisms and asthma have been variable (16–18). To determine whether *ADAM33* gene polymorphisms associate with asthma and, if so, whether the genetic association is stronger in high-severity asthma, we performed a case–control study in the east China Han population.

MATERIALS AND METHODS

Study Subjects

A total of 150 asthmatic patients and 74 healthy controls were enrolled in this study. The diagnosis of asthma met either the NIH Guidelines for the Diagnosis and Management of Asthma (Expert Panel Report 2007) or the GINA (Global Initiative for Asthma) guidelines. All asthma patients were unrelated and were not taking long-term control medications before the enrollment. The asthma patients were further subcategorized into two larger groups according to the percentage of forced expiratory volume in 1 second (FEV₁%) of predicted value, symptoms, nighttime awakening, requirement for short-acting β_2 -agonist, and interference with normal activities. The low-severity group consisted of patients with intermittent or mild persistent asthma attacks. The high-severity group consisted of patients with moderate or severe persistent asthma attacks. The normal controls were recruited from the general population who were receiving regular annual medical examinations and were without symptoms or history of asthma or atopic disease. Also, their first-degree relatives were without history of asthma or atopic disease. All subjects in this study were from the Han population who live in east China. The characteristics of the study subjects are listed in Table 1.

All subjects in this study were recruited from Zhongshan Hospital, Fudan University, in China between August 2007 and June 2008. The Ethics Committee of Fudan University approved the study, and written informed consent was obtained from each individual.

TABLE 1.—Characteristics of study population.

Characteristics	Control	Asthma	
		Low severity	High severity
Number of subjects	74	82	68
Age: median (range)	43.7 (20–69)	37.1 (20–69)	49.8 (13–75)*
Sex (male/female)	32/42	35/47	34/34
Number of smokers (%)	15 (20.3)	6 (7.3)	14 (20.6)
Smoking (pack-year)	15.8 $\pm$ 13.2	14.2 $\pm$ 9.7	21.2 $\pm$ 14.9
FEV ₁ (%predicted)	95.6 $\pm$ 12.8	94.2 $\pm$ 9.6	58.2 $\pm$ 17.0**
Total IgE (IU/ml)	145 $\pm$ 182	192 $\pm$ 327	487 $\pm$ 621**

Notes: Data expressed as mean $\pm$ SD. FEV₁, forced expiratory volume in 1 second. P-values: *P-value < .01, as compared with the low-severity group, **P-value < .01, as compared with control.

Collection of Blood Samples. Shortly after enrollment, 10 ml of whole blood was collected from each individual. Five milliliters of the whole blood was frozen for DNA extraction. The rest was left for 30–60 minutes at room temperature for separation of serum. The serum was collected and frozen at -20°C for measurement of total IgE by ELISA (Pharmacia Diagnostics, Uppsala, Sweden).

DNA Extraction and Genotyping. Genomic DNA was extracted from 5 ml of frozen whole blood using a DNA Extraction Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. We selected six SNPs from the *ADAM33* gene, which had previously been shown to have an allelic and/or haplotype association with asthma, including F + 1 (rs511898), ST + 4 (rs44707), S1 (rs3918396), and S2 (rs528557), T1 (rs2280091), T2 (rs2280090). SNP details and sequence data were obtained through the NCBI databases, <http://www.ncbi.nlm.nih.gov>. The polymorphic region was amplified by polymerase chain restriction (PCR) with a T Gradient Thermoblock PCR System (Biometra, Goettingen, Germany) in a 25 μl reaction solution containing 0.3 μg genomic DNA, 1 $\times$ PCR buffer, 0.3 mM MgCl₂, 0.2 mM dNTPs, 2U TaqDNA polymerase (Takara, Katsushika, Japan), and 0.1 μmol of each primer (Invitrogen, Carlsbad, CA, USA). Genotyping primers and annealing temperature are shown in Table 2. PCR products were digested overnight with restriction enzymes (NEB, Hertfordshire, UK) according to the manufacturer's protocol and analyzed by 2% agarose gel electrophoresis.

Statistical Analysis

We tested each variable for deviations from the Hardy–Weinberg equilibrium by means of the χ^2 -test. The genotype distributions and allelic frequencies were statistically compared between asthmatic patients and controls using the χ^2 -test with SPSS software for Windows (version 13.0; SPSS Inc., Chicago, IL, USA). Linkage disequilibrium between loci was measured using r^2 . Comparisons of the distributions of the allele and genotype frequencies were performed using Fisher's exact test. In order to obtain a measure of significance corrected for multiple testing biases, we ran 10,000 permutations to compute p -values using the Haploview program (Mark Daly's Laboratory, Broad Institute of MIT and Harvard, Cambridge, MA, USA). The relative risk associated with rare alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI).

RESULTS

The characteristics of study population with respect to age, sex, smoking history, serum IgE, and lung function are shown in Table 1. As expected, FEV₁% predicted was significantly decreased in the high-severity group as compared with the low-severity group (58% as compared with 94%, $p < .01$), while serum total IgE was significantly increased in high-severity group

TABLE 2.—Overview of the six SNPs of *ADAM33* gene in our case-control association study.

SNP	ID	Variation	Location	Forward (F) and reverse (R) primers for PCR	Annealing temperature (°C)
F + 1	rs511898	7575 G/A	Intron 6	5'-GTGCTTGAGGGGCTGAGCTG-3'(F) 5'-CACCTAGTGGCCTGATCAC-3'(R)	60
ST + 4	rs44707	11434C/A	Intron 19	5'-AAGGCATGGCCCTCTACCTCCAGT-3'(F) 5'-TGTCTGTTATGCCCTCTGCCCC-3'(R)	60
S1	rs3918396	G/A (Val710Ile)	Exon19	5'-AGGAGTGGGGGTGACCTTGG-3'(F) 5'-GAGGCCACTGGAACCTCCTG-3'(R)	60
S2	rs528557	10918G/C (Gly717Gly)	Exon19	5'-AGGAGTGGGGGTGACCTTGG-3'(F) 5'-GAGGCCACTGGAACCTCCTG-3'(R)	60
T1	rs2280091	12433T/C (Met764Thr)	Exon 20	5'-GTGGCTGCTGGGCTCATGAG-3'(F) 5'-GTGACTTGGAGCAGATGGGTG-3'(R)	55
T2	rs2280090	12462C/T (Pro774Ser)	Exon 20	5'-GTGGCTGCTGGGCTCATGAG-3'(F) 5'-GTGACTTGGAGCAGATGGGTG-3'(R)	55

than in low-severity group (487 IU/ml as compared with 192 IU/ml, $p < .01$). The median age of high-severity group was 49.8, which was significantly older than the other two groups. There were no significant differences in the number of smokers and the length of smoking history between each group. The patients of high-severity group (median 49.8) were older than the other groups.

The distributions of six SNPs were in Hardy-Weinberg equilibrium ($p > .05$) in both groups of asthmatic patients and in normal controls. Genotype frequencies and allele frequencies of six SNPs are shown in Tables 3 and 4, respectively. Among the six types of SNPs of *ADAM33* studied, F1 + 1, T1, and T2 had significant

associations with asthma ($p = .058$, .0067, and .0067, respectively, Table 3). These SNPs were 7575G/A (SNP F + 1) in intron 6 and 12433T/C (SNP T1, Met764Thr) and 12462C/T (SNP T2, Pro774Ser) in exon 20. Furthermore, a comparison of allelic frequencies in these SNPs indicated that the frequencies of common alleles (7575G, 12433T, 12462C) were significantly lower in asthmatic individuals than that in healthy control subjects ($p = .0045-.0072$, OR = 1.81–6.35).

As shown in Tables 3 and 4, the asthmatic patients were divided into two subgroups based on clinical characteristics. For the SNPs F + 1 and T1, the allele frequencies of both the low- and high-severity groups

TABLE 3.—Genotype frequency of six different SNPs of *ADAM33* between patients with asthma and controls.

SNP	Group	<i>n</i>	Genotype frequency			<i>P</i> -value ^a
F + 1			G/G (%)	G/A (%)	A/A (%)	
rs 511898	Asthma	150	72 (48.0)	67 (44.7)	11 (7.3)	.0058
	Low severity	82	39 (47.6)	38 (46.3)	5 (6.1)	.011
	High severity	68	33 (48.5)	29 (42.7)	6 (8.8)	.031
	Control	74	52 (68.1)	18 (24.3)	4 (6.8)	
ST + 4			C/C (%)	A/C (%)	A/A (%)	
rs44707	Asthma	150	47 (31.3)	79 (52.7)	24 (16.0)	.28
	Low severity	82	26 (31.7)	42 (51.2)	14 (17.1)	.38
	High severity	68	21 (30.9)	37 (54.4)	10 (14.7)	.93
	Control	74	31 (41.9)	32 (43.2)	11 (14.9)	
S1			G/G (%)	A/G (%)	A/A (%)	
rs3918396	Asthma	150	130 (86.7)	20 (13.3)	0 (0)	.37
	Low severity	82	74 (90.2)	8 (9.8)	0 (0)	.78
	High severity	68	57 (83.8)	11 (16.2)	0 (0)	.20
	Control	74	68 (91.9)	6 (8.1)	0 (0)	
S2			G/G (%)	C/G (%)	C/C (%)	
rs528557	Asthma	150	92 (61.3)	52 (34.7)	6 (4.0)	.82
	Low severity	82	50 (61.0)	28 (34.1)	4 (4.9)	.92
	High severity	68	43 (63.2)	22 (32.4)	3 (4.4)	.84
	Control	74	43 (58.1)	28 (37.8)	3 (4.1)	
T1			T/T (%)	T/C (%)	C/C (%)	
rs2280091	Asthma	150	118 (78.7)	32 (21.3)	0 (0)	.0067
	Low severity	82	66 (80.5)	16 (19.5)	0 (0)	.033
	High severity	68	52 (76.5)	16 (23.5)	0 (0)	.0081
	Control	74	69 (93.2)	5 (6.8)	0 (0)	
T2			C/C (%)	C/T (%)	T/T (%)	
rs2280090	Asthma	150	127 (84.7)	22 (14.7)	1 (0.7)	.0067
	Low severity	82	73 (89.0)	9 (11.0)	0 (0)	.06
	High severity	68	54 (79.4)	13 (19.1)	1 (1.5)	.0011
	Control	74	72 (97.3)	2 (2.7)	0 (0)	

Note: ^a*P*-values in bold are values from Fisher exact test.

TABLE 4.—Allele frequencies of six SNPs of *ADAM33* between patients with asthma and controls.

SNP	Group	<i>n</i>	Allele frequency		OR (95% CI)	<i>P</i> ^a	<i>P</i> ^b
F + 1			G (%)	A (%)			
rs511898	Asthma	150	211 (70.3)	89 (29.7)	1.81 (1.12–2.92)	.015	.007
	Low-severity	82	116 (70.7)	48 (29.3)	1.94 (1.13–3.33)	.017	.021
	High severity	68	95 (69.9)	41 (30.1)	20.3 (1.16–3.55)	.017	.034
	Control	74	120 (81.1)	28 (18.9)			
ST+4			C (%)	A (%)			
rs44707	Asthma	150	173 (56.7)	127 (42.3)	1.28 (0.85–1.92)	.236	.3
	Low-severity	82	94 (57.3)	70 (42.7)	1.66 (1.05–2.6)	.32	.037
	High-severity	68	79 (58.1)	57 (41.9)	1.92 (0.57–2.50)	.80	1
	Control	74	94 (63.5)	54 (36.5)			
S1			G (%)	A (%)			
rs3918396	Asthma	150	280 (93.3)	20 (6.7)	1.69 (0.66–4.30)	.2665	.75
	Low-severity	82	156 (95.1)	8 (4.9)	1.21 (0.41–3.58)	.79	.86
	High-severity	68	125 (91.9)	11 (8.1)	2.08 (0.75–5.80)	.21	.86
	Control	74	142 (95.9)	6 (4.1)			
S2			G (%)	C (%)			
rs528557	Asthma	150	236 (78.7)	64 (21.3)	0.91 (0.57–1.46)	.6933	1
	Low-severity	82	128 (78)	36 (19)	0.94 (0.55–1.6)	.89	.86
	High-severity	68	108 (79.4)	28 (20.6)	0.87 (0.49–1.53)	.67	.62
	Control	74	114 (77)	34 (20)			
T1			T (%)	C (%)			
rs2280091	Asthma	150	268 (89.3)	32 (10.7)	3.41 (1.30–8.96)	.009	.0072
	Low-severity	82	148 (90.2)	16 (9.8)	3.09 (1.10–8.66)	.039	.038
	High-severity	68	120 (88.2)	16 (11.8)	3.81 (1.34–10.71)	.011	.013
	Control	74	143 (96.6)	5 (3.4)			
T2			C (%)	T (%)			
rs2280090	Asthma		276 (92.0)	24 (8.0)	6.35 (1.48–27.23)	.005	.0045
	Low-severity	82	155 (94.5)	9 (5.5)	4.24 (0.90–19.95)	.065	.069
	High-severity	66	121 (89.0)	15 (11.0)	9.05 (2.03–40.35)	.0007	.0008
	Control	74	146 (98.7)	2 (1.3)			

Notes: ^a*P*-values in bold are values from Fisher exact test.^b*P*-values in bold are permutation adjusted.

were significantly different from those in the control group ($p = .013-.038$). For the SNP T2, the difference in allelic distribution was only observed between the high-severity and control groups ($p = .0081$). No statistically significant differences in allele and genotype frequencies between the low-severity and control groups were observed.

We further analyzed the haplotype structure using three SNPs (7575G/A [SNP F + 1], 12433T/C [SNP T1], and 12462C/T [SNP T2]). There were seven haplotypes in all samples. Estimated frequencies of the three-locus

haplotypes were compared between asthma and control subjects. We found that the most common haplotype was 7575G, 12433T, and 12462C (GTC haplotype) in both groups (see Table 5). This haplotype was more frequently observed in healthy controls than in asthmatic patients (78.4% vs. 61.8%, $p = .0004$). Haplotype GTC frequency in the control group was also significantly higher than in the high- or low-severity asthmatic groups. We did not find any significant difference of other haplotype frequencies between asthma patients and controls.

TABLE 5.—*ADAM33* haplotype profiles in our study population.

	SNP position			Estimated frequency	Haplotype frequency		<i>P</i> -value ^a	Permutation <i>P</i> -value ^b
	F + 1	T1	T2		Cases	Controls		
Haplotype 1	G	T	C	0.673	0.618 0.644 (low) 0.589 (high)	0.784 0.784 0.784	.0004 .0066 .0004	.001 .024 .001
Haplotype 2	A	T	C	0.209	0.228	0.169	.15	.57
Haplotype 3	G	C	C	0.039	0.044	0.027	.37	.94
Haplotype 4	G	T	T	0.023	0.028	0.014	.33	.92
Haplotype 5	A	C	C	0.022	0.029	0.007	.12	.53
Haplotype 6	A	C	T	0.014	0.021	0	.08	.33
Haplotype 7	A	T	T	0.012	0.019	0	.09	.39

Notes: Low, the low-severity group; High, the high-severity group.

^a*P*-values in bold are values from Fisher exact test.^b*P*-values in bold are permutation adjusted.

DISCUSSION

In this study, we have found that three SNPs of *ADAM33* associate with asthma susceptibility in the east China Han population. They are SNP F + 1 (rs511898, 7575G/A) located in intron 6, SNP T1 (rs2280091, 12433T/C) in exon 20, and SNP T2 (rs 2280090, 12462C/T) in exon 20. In addition, SNPs T1 and T2 exhibited stronger genetic association in the high-severity group of asthmatic patients than in the low-severity group when both groups are compared with healthy controls.

Previous studies from other investigators have also shown that some of the six SNPs we used in this study are associated with asthma and BHR in ethnically diverse populations. For example, SNPs F + 1, ST + 4, S1, S2, T1, and T2 are related to Caucasian populations from the United Kingdom or the United States (2). Also, some of the SNPs are related to African Americans, US whites and US Hispanics (9), Germans (10), and Colombians (19). In Asia, some of the six SNPs are associated with asthma in Indian (20), Japanese (11), Thai (21), and Korean (12) populations. In China, Wang et al. (16) first genotyped three SNPs of *ADAM33* (F + 1, S + 1, and T1) in a case-control study and found that there was no significant association between these SNPs and asthma susceptibility. However, both Qiu et al. (17)

and Su et al. (18) found that some SNPs of *ADAM33* may correlate with susceptibility to asthma in the Han population of south (T1) and northeast China (V4, T2, T1, and Q-1). Evidence from our study also shows that SNPs F + 1, T1, and T2 of *ADAM33* are associated with asthma while SNPs ST + 4, S1, and S2 are not. In order to compare results from different investigators, we conducted a literature review as shown in Table 6. The differences in observations might be explained by the variability in sample sizes, heterogeneity of the study populations, and environmental exposure. Even in the same ethnic Han population, there is considerable genetic, linguistic, cultural, and social diversity among the subgroups. Our study further demonstrates that no single SNP universally associates with the asthma phenotype in ethnically diverse populations.

Since *ADAM33* is preferentially expressed in smooth muscle, myofibroblasts, and fibroblasts in the lung (2), alterations in *ADAM33* activity may underlie abnormalities of airway smooth muscle cells or fibroblasts, which further leads to airway remodeling and progressive decline of lung function in asthma. Because of this, we analyzed the association of *ADAM33* SNPs with asthma severity by dividing the asthmatic patients into high- and low-severity groups. We found that the allele frequencies of F + 1 and

TABLE 6.—Comparison of previously reported association results with *ADAM33* polymorphisms.

Year	Study	Study population cc = case-control fa = family study	N2 Cases/controls Families (ind.)	<i>ADAM33</i> SNPs and reported associations
European and American				
2002	Van Eerdewegh et al. (2)	US/UK combined (cc)	130/217	Q-1; S1; ST + 4; ST + 7; V-1; V4
		UK (cc)	(not reported)	F + 1; Q-1; S1; S2; ST + 4; V-1; V4
		US (cc)	(not reported)	L-1; M + 1; T1; T2; T + 1
		US/UK (fa)	460fa (1840)	S1; T1
2003	Lind et al. (14)	Mexican (cc)	190/160	Negative
		Puerto Rican (cc)	183/165	Negative
		Mexican/Puerto Rican (fa)	583fa (1749)	Negative
2004	Raby et al. (15)	Non-Hispanic white (fa)	474 (1462)	Negative
		Hispanic (fa)	47 (149)	T1; T + 1
		African American (fa)	66 (203)	Negative
2004	Werner et al. (10)	German (cc)	48/499	ST + 5; ST + 7
		German (fa)	171fa (732)	F + 1; S2; ST + 4; ST + 5
2003	Howard et al. (9)	African American (cc)	161/265	S2; ST + 4; V4
		US white (cc)	220/229	S2; ST + 7; T1; T2
		US Hispanic (cc)	113/127	S2; ST + 4; T1; T2; V-1
		Dutch white (cc)	180/133	S1; S2; ST+7; V-1; V4
2006	Schedel et al. (22)	German (cc)	1872/824	Negative
2010	Vergara et al. (19)	Colombia (cc)	429/401	ST + 7; V4; T2; T1
Asian				
2004	Lee et al. (12)	Korean (cc)	326/151	T1
2006	Hirota et al. (11)	Japanese (cc)	504/651	T1; T2; S2; V3
2006	Wang et al. (16)	Chinese	296/270	Negative
2007	Qiu et al. (17)	South China Han population (cc)	160/95	T1
2008	Su et al. (18)	Chinese Han population	181/151	V4; T2; T1; Q-1
2010	Awasthi et al. (20)	Indian children (cc)	211/137	S2; ST + 5; F + 1; ST + 4; V4
Association with asthma severity				
2004	Jongepier et al. (13)	Dutch Caucasian	200	S2 (excess decline in FEV ₁)
2006	Kedda et al. (23)	Australian Caucasian (cc)	612/473	Negative
		Mild, moderate, and severe	292/238/82	Global haplotypic (V-1 and ST + 7) with asthma severity
2008	Thongngarm et al. (21)	Thai (cc)	200/100	S2 (low-severity group); ST + 4 (high-severity group)
2008	de Faria et al. (24)	Spain	88 (202)	Negative with severity (S2)

T1 in both asthmatic groups were significantly different from healthy controls (Tables 3 and 4). For the SNP T2, the difference in genotype and allelic frequency was only significant between high-severity group and controls. This result suggests that the SNP T2 might be linked to asthma severity. The genetic association of T2 with asthma is stronger in high-severity asthmatics than in low-severity asthmatics ($p = .0011$ vs. $p = .06$). This finding was in accordance with previous studies that indicate that *ADAM33* SNPs associate with lung function decline in asthmatic subjects, of which three SNPs (S2, T1, T2) demonstrated an association with excess FEV₁ decline (13). It is important to note that *ADAM33* SNPs have also shown an association with excess FEV₁ decline in a general population sample ($n = 1390$) followed for 25 years. The main SNPs identified included Q-1, S1, and S2, with excess decline of 23.6–9.6 ml/year (25). However, other studies did not show any association with asthma severity (23, 24). Further analysis demonstrates that the frequency of rare alleles of T1 (T1 C) or T2 (T2 T) was increased in asthmatic patients compared with normal controls which corresponds with other studies in ethnically diverse populations, such as US white (9), Korean (12), Japanese (11), and Chinese populations (17, 18). This could have some association with the pathogenesis of asthma since T1 and T2 are located in the cytoplasmic domain of *ADAM33* protein. Changes in alleles of SNPs T1 and T2 could potentially alter intracellular signaling, resulting in increased fibroblast and smooth muscle proliferation that leads to airway remodeling. However, further study is required to elucidate precisely which SNPs in *ADAM33* alter the gene expressions and/or the function of its product.

In addition to the analysis of genotype and allele frequency, we further analyzed the haplotype structures using three SNPs: F + 1 (7575G/A), T1 (12433T/C), and T2 (12462C/T). We found that the frequency of the haplotype GTC (7575G, 12433T, and 12462C) was significantly higher in the control group than in both groups of asthmatics (78.4% vs. 61.8%, Table 6). This may imply that the GTC haplotype is a protective or resistant allele for asthma. The association studies between asthma and *ADAM33* haplotypes have been variable so far. Haplotype H4 (GCAGGG) was found to be strongly associated with asthma in a family-based analysis of population in the Caribbean coast of Colombia (19). Haplotype Ht4 (GCGG) showed a significant association with BHR in Korean asthmatics (12). A two-SNP haplotype (in V-1 and ST + 7) is found to be significantly associated with asthma and even disease severity in a large Australian Caucasian population (23). Haplotype association with BHR and asthma was also found in the UK and US populations (2). However, haplotype H4 did not show any influence on BHR or only a minor influence on asthma in a German population (22). Haplotype TCC (in T1/V-1/V4) was not found related to asthma in Puerto Ricans and Mexicans (14). None of the six SNP haplotypes was associated with asthma

susceptibility in Dutch, African American, white, and Hispanic populations (9). The remarkable diversity in haplotype and frequency of *ADAM33* may further explain the discrepancies in different populations and also in different subgroups within the same population.

Taken together, our results suggest that polymorphisms in the *ADAM33* gene SNPs F + 1, T1, and T2 associate with asthma in an east China Han population. In addition, the T2 SNP of *ADAM33* shows a stronger genetic association with high-severity asthma. Thus, our study further demonstrates that polymorphisms of *ADAM33* gene play a role in asthma susceptibility and severity.

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DECLARATION OF INTEREST

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