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Association between polymorphisms in *ADAM33*, *CD14*, and *TLR4* with asthma in the Uygur population in China

J. Wang[1,2], M. Simayi[1], Q. Wushouer[1], Y. Xia[1], Y. He[1], F. Yan[1], J. Zhang[3], S. Cui[1] and H. Wen[4]

[1]Department of Respiratory Medicine,
The First Affiliated Hospital of Xinjiang Medical University,
Urumqi, Xinjiang, China
[2]Mobile Postdoctoral Station,
The First Affiliated Hospital of Xinjiang Medical University,
Urumqi, Xinjiang, China
[3]Department of Pathophysiology, Xinjiang Medical University,
Urumqi, Xinjiang, China
[4]Xinjiang Key Laboratory of Echinococcosis and Medical Research Center,
The First Affiliated Hospital of Xinjiang Medical University,
Urumqi, Xinjiang, China

Corresponding author: H. Wen
E-mail: haowen59@163.com

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ABSTRACT. We evaluated the associations between single nucleotide polymorphisms (SNPs) and haplotypes of the genes encoding a disintegrin and metalloproteinase 33 (*ADAM33*), cluster of differentiation 14 (*CD14*), and Toll-like receptor 4 (*TLR4*) and the susceptibility of developing specific adult phenotypes of bronchial asthma in a Chinese Uygur population. Five SNPs of *ADAM33* (T1, T2, and V4), 3 SNPs of *CD14* (-1359G/T, -1145G/A, and -159T/C), and 2 SNPs of *TLR4* (-896A/G and -1196C/T) were genotyped in a Chinese Uygur sample of 126 adult asthmatic patients and

126 control subjects. Gene polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism analysis. The genotyping results were confirmed in a random subgroup of our samples using direct DNA sequencing. The allele frequencies of *ADAM33* T1 (TC), T2 (AG), and V4 (GG) were significantly higher in patients than in controls ($P < 0.05$). The genotypes T1 (TC+CC), T2 (AG+AA), and V4 (CG+GG) significantly increased the risk of asthma. After adjusting for confounding factors, these associations were stronger and remained significant. The T1 (TC) and V4 (GG) genotypes in the *ADAM33* gene were associated with significantly decreased FEV1 levels in patients with asthma. The haplotype frequencies of Hap3 (CAC) and Hap4 (CAG) were significantly higher in patients than in controls ($P < 0.05$). Our results suggest that polymorphisms T1, T2, and V4 in *ADAM33* may contribute to the susceptibility to asthma. Specific haplotypes of *ADAM33* may contribute to a higher susceptibility to asthma in the Chinese Uygur population.

Key words: ADAM33, CD14, TLR4, Asthma, Polymorphisms, Uygur population

INTRODUCTION

Asthma is a complex disease that is caused by both environmental and genetic factors. The disintegrin and a disintegrin and metalloproteinase 33 (ADAM33) gene, the first asthma candidate gene identified by positional cloning, has been associated with asthma, chronic obstructive pulmonary disease (COPD), and bronchial hyperresponsiveness (BHR) (Sadeghnejad et al., 2009; Vergara et al., 2010). ADAM33 is a complex molecule whose expression is largely restricted to mesenchymal cells, including smooth muscle cells of the bronchia and pulmonary fibroblasts, which plays an important role in airway remodeling (Puxeddu et al., 2008).

A functional single nucleotide polymorphism (SNP) in the 5' genomic region of the cluster of differentiation 14 gene (*CD14*) is one of the most widely tested genetic variations for its relationship with asthma and other traits (Choudhry et al., 2005). *CD14* expression was found to increase in asthmatics after lipopolysaccharide (LPS) inhalation (Alexis et al., 2001). Altered *CD14* expression may affect the proportion of Th1 to Th2 cells, thereby influencing IgE responses (Baldini et al., 1999) and associated inflammatory phenotypes in allergic conditions such as asthma.

Toll-like receptor 4 (TLR4) is a recently identified transmembrane protein that can specifically detect LPS, a component of the cell wall of Gram-negative bacteria, and is closely associated with the morbidity of many diseases, including asthma, pyaemia, and coronary disease (Yang et al., 2004; Schoneveld et al., 2008). According to previous studies, 2 common gene polymorphism sites are present in the extracellular region of *TLR4*, at positions -896 (A→G) and -1196 (C→T), which alter the amino acids 299 and 399, respectively, and reduce the protein's sensitivity to LPS, thereby facilitating infection with Gram-negative bacteria. Yamashita and Nakayama (2008) showed that TLR4 plays an important role in allergic reactions in the respiratory tract.

Different genetic backgrounds and environmental exposures in different ethnic popu-

lations may affect the pathogenesis of asthma. Therefore, asthma susceptibility genes in different populations may also differ. To date, most available data for the relationship between *ADAM33* and asthma have been obtained from Caucasian, Japanese, and Chinese Han populations. The Uygur population of Northwest China, comprises approximately 11,000,000 people. Places of residence are relatively stable in the Uygur population and there is little migration. Uygurs are rarely associated with ethnic intermarriages and their lifestyles are consistent. Therefore, the Uygur population is an ideal population to conduct genetic studies. The Uygur population is an admixture of European (40-60%) and East Asian ancestry, and thus differs from other Chinese populations. We investigated genetic polymorphisms thought to be associated with asthma in other populations in this unique population. The purpose of the present study was to evaluate the associations between genetic variants of *ADAM33*, *CD14*, and *TLR4* and asthma as well as asthma-related phenotypes in the Chinese Uygur population.

MATERIAL AND METHODS

Study subjects

A total of 126 unrelated adult patients with asthma (66 males and 60 females) were recruited from the outpatient department of Turpan Prefecture Hospital, Xinjiang, China. The average age of the patients was 41.1 years, ranging from 16 to 79 years. In addition, 126 control subjects were recruited from the same hospital. The average age of the controls was 40.6 years, ranging from 18 to 78 years. The controls had no history of asthma, rhinitis, or other chronic pulmonary diseases and were matched with patients by age (± 5 years), gender, ethnic origin, and residential area. All study subjects were Chinese Uygurs who lived in Xinjiang, China. Informed consent was obtained from all participating subjects. All study procedures for all participants were approved by the Institutional Review Boards of The First Affiliated Hospital of Xinjiang Medical University.

Cases and controls were interviewed by investigators using the same structured questionnaire. The serum-eosinophil cationic protein level (S-ECP), total immunoglobulin E (T-IgE), and specific immunoglobulin E (S-IgE) were measured using the CAP system (Pharmacia Diagnostics; Uppsala, Sweden). S-IgE levels were determined for house dust, *Dermatophagoides farinae*, *Artemisia*, *Betula alba*, mold (multivalent), insects (multivalent), cat dander, and dog dander. Values of 0.35 U/mL or greater, corresponding to a radioallergosorbent (RAST) score $\geq$1, were considered positive. The severity of adult asthma was classified according to the criteria of the Global Initiative for Asthma (GINA), and was defined according to the degree of therapy required to control symptoms at the time of entry into the study. Among the 126 patients, asthma in 62 (49%) patients was mild, intermittent, and persistent, in 39 (31%) patients was moderate and persistent, and in 25 (20%) patients was severely persistent. All patients met the following criteria: i) no history of diseases in relatives, such as immune system diseases; ii) no severe excitable state such as severe infection, new-onset myocardial infarction, or stroke; and iii) long-term residence in the Xinjiang area (third generation or more).

Data collection and definition of risk factors

Information regarding demographic characteristics and other risk factors was collected

by use of a structured questionnaire involving the following risk factors: history of allergies, family history, body mass index (BMI), cigarette smoking, alcohol drinking, place of residence (countryside or city), and occupation. White blood cell counts and chest X-rays were obtained. The family history was obtained and first-degree relatives of subjects diagnosed with asthma were noted. If a subject had smoked at least 1 cigarette per day for at least 1 year and had not quit smoking by the time of participation in the study, he/she was defined a current smoker.

DNA extraction and genotyping

Genomic DNA was extracted from 5 mL frozen whole blood using a DNA extraction kit (Qiagen; Hilden, Germany) according to the manufacturer protocol. DNA samples were stored at -80°C. Primer sequences, restriction enzymes, and sizes of fragments generated by the *ADAM33*, *CD14*, and *TLR4* genetic polymorphisms are described in Table 1.

Table 1. Primer sequences, restriction enzymes and sizes of fragments generated by ADAM33, CD14 and TLR4 gene polymorphisms.

Gene	SNP	Variation	Location	SNP name*	Forward (F) and reverse (R) primers for PCRw	Amplified fragment (pb)	Restriction enzyme	Length of digested fragments
ADAM33	rs2280091	12433T/C (Met764Thr)	Exon 20	T1	F: 5'-AGAGGGTGACTTGGAGCAGA-3' R: 5'-CCAGAAACCTGATTAGGGGG-3'	576	Nco I	A:140 + 260 G:400
ADAM33	rs2280090	12462C/T (Pro774Ser)	Exon 20	T2	F: 5'-TTCTCAGGGTCTGGGAGAAA-3' R: 5'-GCCAACCTCCTGGACTCTTA-3'	310	HpyCH4 III	A:198 +112 G:310
ADAM33	rs2787094	13506C/G	Exon 22	V4	F: 5'-ACACACAGAATGGGGGAGAG-3' R: 5'-CCAGAAGCAAAGGTCACACA-3'	374	Pst I	G:168 + 206 C:374
CD14	rs3138078	-1359G/T	Promoter		F: 5'-CTCAGGAATCTGAGGCAAGA-3' R: 5'-AGTACAATCTCTGTGCCCTA-3'	371	Fok I	G: 192+178 T: 192+112+66
CD14	rs2569191	-1145G/A	Promoter		F: 5'-CTCAGGAATCTGAGGCAAGA-3' R: 5'-AGTACAATCTCTGTGCCCTA-3'	371	HpyCH4V	A: 230+71+70 G: 300+71
CD14	rs2569190	-159T/C	Promoter		F: 5'-GTGCCAACAGATGAGGTTCAC-3' R: 5'-GCCTCTGACAGTTTATGTAATC-3'	497	Ava II	C: 497 T: 353+144
TLR4	rs4986790	-896A/G (AsP299Gly)	Exon 4		F: 5'-GATTAGCATACTTAGACTACTACCTCCATG-3' R: 5'-GATCAACTTCTGAAAAAGCATTCC CAC-3'	249	Not I	A: 249 G: 223
TLR4	rs4987223	-1196C/T (Thr399Ile)	Exon 4		F: 5'-GGTTGCTGTTCTCAAAGTGATTTTGGGAGA A-3' R: 5'-CCTGAAGACTGGAGAGTGAGTTAAATGCT-3'	406	Hinf I	T: 406 L: 377

*In reference Van Eerdewegh et al. (2002).

Statistical analysis

All continuous variables are reported as the means ± SD unless otherwise indicated. The χ^2 test was used to examine the deviation of genotype distributions from Hardy-Weinberg equilibrium. The χ^2 test or Fisher's exact test was used to examine the association between the SNPs and asthma. Analysis of variance was used for evaluating quantitative traits. The Haploview 3.2 software was used to reconstruct haplotypes and to estimate haplotype frequencies in cases and controls. Multivariate conditional logistic regression analysis was performed to assess the independent roles of the *ADAM33* and *CD14* genotypes and other risk factors. Odds ratios (ORs) and 95% confidence intervals (95%CI) were calculated. All statistical tests were performed using the SPSS17.0 software for Windows (SPSS, Inc.; Chicago, IL, USA). The haploid frequency distributions were determined using the linkage disequilibrium online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php). The inspection level for evaluating statistical significance was $\alpha = 0.05$.

RESULTS

Characteristics of study subjects

As shown in Table 2, there was no significant difference in age, residence, or cigarette smoking between patients and controls. However, there was a significant difference in BMI between the 2 groups ($P < 0.001$), suggesting that BMI is a risk factor for asthma. Spirometric assessment was performed on all study subjects, revealing a lower predicted FEV1% in cases compared to controls (77.52 ± 12.78 *vs* 98.21 ± 7.45, $P < 0.001$). T-IgE and S-ECP levels were significantly higher in patients with asthma than in controls (91.46 ± 29.28 *vs* 43.52 ± 13.46, and 6.67 ± 1.45 *vs* 2.28 ± 1.09, $P < 0.001$, respectively). Asthma patients showed much more positive S-IgE levels than did controls (30.95% *vs* 7.14%, $P < 0.001$). The frequency of conventional risk factors for asthma, including family history of asthma and history of allergies, was higher in the patient group than in the control group ($P < 0.001$).

Table 2. Demographic characteristics and distribution of risk factors.

Characteristic	Asthma (%)	Control (%)	P
Number of patients	126	126	
Gender (N, %)			
Male	66 (52.38)	66 (52.38)	
Female	60 (47.62)	60 (47.62)	
Age (years ± SD)	41.06 ± 9.75	40.58 ± 9.24	0.542
Living place			
City	65 (51.60)	58 (46.03)	0.778
Countryside	61 (48.40)	68 (53.97)	0.778
BMI (kg/m^2)	25.87 ± 1.90	24.12 ± 2.01	<0.001
Cigarette smoking	34 (26.98)	30 (23.81)	0.563
Family history of asthma	29 (23.02)	5 (3.97)	<0.001
History of allergy	44 (34.92)	15 (11.90)	<0.001
Positive S-IgE	39 (30.95)	9 (7.14)	<0.001
Total IgE (kU/L ± SD)	91.46 ± 29.28	43.52 ± 13.46	<0.001
Serum S-ECP (μg/L ± SD)	6.67 ± 1.45	2.28 ± 1.09	<0.001
FEV1% predicted (% ± SD)	77.52 ± 12.78	98.21 ± 7.45	<0.001

Association of SNPs in *ADAM33*, *CD14*, and *TLR4* with asthma

All SNPs conformed to Hardy-Weinberg equilibrium in the patient group and control group. Among the 5 studied SNPs in *ADAM33*, the minor allele frequencies of T1 (C), T2 (A), and V4 (G) were significantly higher in patients than in controls ($P = 0.003$, OR = 2.08; $P = 0.008$, OR = 2.14; and $P < 0.001$, OR = 1.91, respectively; Table 3). The genotype frequencies of T1 (TC), T2 (AG), and V4 (GG) were higher in patients than in controls ($P = 0.016$, OR = 2.12; $P = 0.034$, OR = 1.93; and $P = 0.022$, OR = 1.86, respectively). The genotypes T1 (TC+CC), T2 (AG+AA), and V4 (CG+GG) increased the risk of asthma ($P = 0.005$, OR = 2.24; $P = 0.011$, OR = 2.16; and $P = 0.001$, OR = 2.63, respectively). After adjusting for confounding factors, these associations remained significant and were stronger (Table 4). None of the SNPs in *CD14* were significantly associated with asthma ($P > 0.05$; Tables 3 and 4). The minor alleles of the 2 *TLR4* SNPs were not detected in cases and controls in this study (Tables 3).

Table 3. Association of single SNPs in *ADAM33*, *CD14* and *TLR4* with asthma.

Gene	SNP	N	Genotype frequency (%)					Allele frequency (%)		
			Genotype			χ^2	P value	Minor allele	Allele P	OR (95%CI)
ADAM33	12433 T/C (T1)		TT	TC	CC			C		
	Cases	126	64.29	30.16	5.56	8.064	0.018	20.63	0.003	2.080 (1.273-3.398)
	Controls	126	80.16	17.46	2.38			11.11		
	12462C/T (T2)		GG	AG	AA			A		
	Cases	125	69.60	28.0	2.40	8.013	0.018	16.40	0.008	2.139 (1.224-3.739)
	Controls	125	83.20	16.80	0			8.40		
	13506C/G (V4)		GG	GC	CC			G		
	Cases	125	40.0	42.40	17.60	11.737	0.003	61.20	<0.001	1.912 (1.340-2.730)
	Controls	125	26.40	37.60	36.0			45.20		
CD14	-1359G/T		GG	GT	TT			T		
	Cases	126	57.14	38.10	4.76	0.182	0.913	23.81	0.679	0.918 (0.612-1.377)
	Controls	126	54.76	39.68	5.56			25.40		
	-1145G/A		AA	AG	GG			G		
	Cases	126	14.29	46.03	39.68	0.444	0.801	62.70	0.522	1.124 (0.786-1.607)
	Controls	126	15.87	48.41	35.71			59.92		
	-159T/C		CC	CT	TT			T		
	Cases	126	18.25	49.21	32.54	0.104	0.949	57.14	0.787	1.050 (0.737-1.496)
	Controls	126	19.84	48.41	31.75			55.95		
TLR4	-896A/G (AsP299Gly)		AA	AG	GG			G		
	Cases	126	126	0	0			0		
	Controls	126	126	0	0			0		
	-1196C/T (Thr399Ile)		CC	CT	TT			T		
	Cases	126	126	0	0			0		
	Controls	126	126	0	0			0		

Table 4. Odds ratio of different *ADAM33* and *CD14* genotypes for asthma.

Gene	Gentype	Crude OR	95%CI	P	Adjusted OR	95%CI[a]	P
ADAM33							
12433 T/C	TT	1.00[b]					
(T1)	TC	2.118	1.138-3.943	0.016	2.317	1.102-4.870	0.031
	CC	2.307	0.648-8.213	0.192	2.678	0.589-12.184	0.209
	TC+CC	2.244	1.279-3.938	0.005	2.522	1.259-5.055	0.014
12462 C/T	GG	1.00[b]					
(T2)	AG	1.926	1.046-3.545	0.034	2.174	1.011-4.675	0.046
	AA			0.081			0.101
	AG+AA	2.163	1.190-3.930	0.011	2.362	1.090-5.120	0.029
13506 C/G	CC	1.00[b]					
(V4)	CG	1.222	0.736-2.028	0.439	1.365	0.550-3.387	0.462
	GG	1.859	1.089-3.173	0.022	2.071	1.062-4.040	0.035
	CG+GG	2.634	1.477-4.696	0.001	2.949	1.269-6.855	0.012
CD14							
-1359G/T	GG	1.00[b]					
	GT	0.949	0.638-1.411	0.784	0.897	0.386-2.082	0.812
	TT	0.865	0.319-2.348	0.761	0.796	0.162-3.907	.0.788
	GT+TT	0.908	0.553-1.492	0.703	0.862	0.377-1.970	0.726
-1145G/A	AA	1.00[b]					
	AG	0.931	0.643-1.349	0.686	0.874	0.413-1.850	0.707
	GG	1.178	0.719-1.931	0.527	1.010	0.967-1.055	0.551
	AG+GG	1.132	0.568-2.257	0.725	0.922	0.501-1.698	0.749
-159T/C	CC	1.00[b]					
	CT	1.046	0.521-2.100	0.803	1.059	0.395-2.836	0.845
	TT	1.052	0.502-2.206	0.791	1.071	0.357-3.209	0.822
	CT+TT	1.108	0.592-2.073	0.784	1.195	0.371-3.852	0.807

[a]Adjusted for family history of asthma (0 = negative; 1 = positive), history of allergy (0 = negative; 1 = positive), positive S-IgE (0 = negative; 1 = positive), BMI, total IgE, S-ECP, and FEV1% predicted. [b]Reference group.

Association of the *ADAM33* SNPs with FEV1%, T-IgE, and S-ECP levels in patients with asthma

There was a significant difference in the FEV1 level between the TT, TC, and CC genotypes of T1 (P = 0.005). Patients who carried the TC or CC genotype of T1 showed a lower FEV1 level than those with the TT genotype (72.1 or 75.0 *vs* 80.7%). Similarly, a significant difference was observed in the FEV1 level between the CC, CG, and GG genotypes of V4 (P = 0.005). Patients who carried the GG genotype of V4 showed a lower FEV1 level than those with the CG or CC genotype (72.8 *vs* 81.1 or 75.2%). None of the 3 SNPs in *ADAM33* was significantly associated with T-IgE and S-ECP levels in patients with asthma (P > 0.05; Table 5). None of the 3 SNPs in *CD14* was significantly associated with FEV1, T-IgE, and S-ECP levels in patients with asthma (data not shown).

Table 5. Association of the *ADAM33* SNPs with FEV1%, T-IgE, and S-ECP levels in patients with asthma.

Gene	Genotype	N	FEV1% (Means ± SD, %)	T-IgE (kU/L)	S-ECP (μg/L)
ADAM33					
12433 T/C	TT	81	80.74 ± 12.85	89.14 ± 29.21	6.48 ± 1.42
(T1)	TC	38	72.12 ± 14.47	94.86 ± 30.25	7.16 ± 1.51
	CC	7	74.96 ± 12.08	90.23 ± 29.18	6.39 ± 1.38
	F value		5.546	1.079	1.505
	P value		0.005	0.344	0.226
12462 C/T	GG	87	78.05 ± 12.81	90.85 ± 28.84	6.45 ± 1.43
(T2)	AG	35	74.66 ± 12.63	92.57 ± 30.12	7.04 ± 1.52
	AA	3	73.47 ± 13.06	93.42 ± 29.08	6.72 ± 1.39
	F value		2.258	0.825	0.401
	P value		0.085	0.456	0.638
13506 C/G	CC	22	75.17 ± 12.42	88.02 ± 28.24	6.47 ± 1.44
(V4)	CG	53	81.06 ± 13.61	91.24 ± 28.75	6.40 ± 1.41
	GG	50	72.84 ± 12.88	93.15 ± 30.52	7.06 ± 1.50
	F value		5.497	1.724	1.615
	P value		0.005	0.151	0.189

Linkage disequilibrium and haplotype frequencies of *ADAM33* SNPs

Three SNPs in *ADAM33* were located in one extended linkage disequilibrium (LD) block (Table 6). In addition to the strong LD between the T1 and T2 polymorphisms, weak LD between the other SNPs was observed. Four common haplotypes were identified in both the case and control groups (Table 7). A significant difference in haplotype frequency between cases and controls was observed (omnibus P = 0.002). The frequencies of Hap3 (CAC) and Hap4 (CAG) were significantly higher in patients than in controls (P = 0.033, OR = 2.190 and P = 0.036, OR = 2.533, respectively). The frequency of Hap2 (TGC) was significantly lower in patients than in controls (P = 0.001, OR = 0.535).

DISCUSSION

ADAM33 was initially identified as an asthma-susceptible gene using positional cloning (Van Eerdewegh et al., 2002). In recent years, association studies have demonstrated the important role of genetics in bronchial asthma. Polymorphisms of *ADAM33* were originally shown to be associated with BHR. The association between *ADAM33* and asthma and related

phenotypes has been identified in East Asian populations such as the Japanese, Korean, and Chinese Han populations. Substituting thymine for cytosine at the T1 site changes methionine to threonine in the cytoplasmic domain of the protein. This could potentially alter intracellular signaling, resulting in increased fibroblast and smooth muscle cell proliferation. In the present study, the minor allele C of T1 was observed more frequently in patients with asthma than in controls (20.6 *vs* 11.1%; Table 3). Our results are consistent with those reported in a Caucasian population (24.1 *vs* 7.4%), Japanese population (15.3 *vs* 5.3%), and Korean population (9 *vs* 8%) (Van Eerdewegh et al., 2002; Lee et al., 2004; Noguchi et al., 2006). In this study, significant associations were identified between 3 SNPs (T1, T2, and V4) in *ADAM33* and asthma in the Chinese Uygur population (Table 3). Similar associations have been reported for the Japanese population (T1, T2, S2, and V-3) (Hirota et al., 2006) and the Korean population (S1, T1, V-1, V1, and V4) (Lee et al., 2004). A study of the Chinese Han population living in Northern China showed that 4 SNPs (V4, T2, T1, and Q-1) were associated with asthma and allergic rhinitis. A meta-analysis showed that the T1 SNP was associated with asthma in the Chinese population (Li et al., 2010). Another meta-analysis found that the T1 SNP confers susceptibility to asthma in Asians, but no association was observed between the *ADAM33* T2 and ST+7 polymorphisms and asthma susceptibility. Howard et al. (2003) reported an association between *ADAM33* and asthma in 4 distinct cohorts, including the US Caucasian population (ST17, T1, and T2), African American population (S2), US Hispanic population (S2 and T2), and Dutch (ST17 and V4) population. Interestingly, the most significant SNP in the Dutch asthma population was found to be V4; however, this result could not be replicated in the other 3 populations. In the present study, we also found a significant association between SNP V4 and asthma (Table 3). However, in a study of 290 patients and 270 controls in a Chinese Han population, Wang et al. (2006) found no significant association between T1, F+1, and S+1 SNPs and bronchial asthma.

Table 6. Pairwise linkage disequilibrium coefficients between the five SNPs in the *ADAM33* gene.

SNP	T1	T2	V4
T1	-	0.892	0.090
T2	1.000	-	0.106
V4	0.738	0.357	-

D' value (abs) is listed in lower left triangle, and r^2 in upper right.

Table 7. Haplotype frequencies of the *ADAM33* SNPs in patients with asthma and controls.

Haplotype	SNP position			Haplotype frequency		χ^2	P	OR (95%CI)
	T1	T2	V4	Cases (N, %)	Controls (N, %)			
Hap1	T	G	G	98 (39.20)	79 (31.60)	3.157	0.076	1.396 (0.966-2.017)
Hap2	T	G	C	74 (29.60)	110 (44.0)	11.145	0.001	0.535 (0.371-0.772)
Hap3	C	A	C	24 (9.60)	12 (4.80)	4.545	0.033	2.190 (1.052-4.563)
Hap4	C	A	G	17 (6.80)	7 (2.80)	4.377	0.036	2.533 (1.031-6.219)

Omnibus P = 0.002.

Increased expression of the *ADAM33* gene has been observed in patients with more severe asthma (Foley et al., 2007), suggesting that this gene contributes to remodeling during asthma progression. This also indicates that *ADAM33* is a key molecule in the development and progression of asthma and contributes to smooth muscle and vascular modeling. In addi-

tion, recent studies have shown that SNPs in *ADAM33* are associated with accelerated lung function decline in the general population and act as a risk factor for chronic obstructive pulmonary disorder (van Diemen et al., 2005). Therefore, ADAM33 may contribute to accelerated airway dysfunction in various airway diseases. We identified a significant difference in FEV1 levels between different T1 and V4 genotypes (Table 5). In a prospective cohort study of 17 polymorphisms in the *ADAM33* gene, Simpson et al. (2005) found an association between *ADAM33* and asthma, and showed that polymorphisms in the *ADAM33* gene could be used to predict early-stage lung failure. They also found that 4 SNPs (Tl, T2, F+1, and M+1) were associated with decreased FEV1 levels in children at the age of 5 years (Simpson et al., 2005). In the present study, we observed no significant difference in the levels of total serum IgE and ECP between different T1, T2, and V4 genotypes (Table 5), suggesting no association between *ADAM33* and allergies. Our results agree with the findings of Umland et al. (2003), which showed that mRNA of *ADAM33* was highly expressed in smooth muscle-containing organs, and was minimally expressed in immune organs, suggesting a role for *ADAM33* in remodeling rather than in immune responses.

As multiple SNPs may act in concert to increase asthma risk, haplotypes are thought to carry information about unidentified causal variants in the region (Noguchi et al., 2006). We found that SNP T2 was in strong LD with T1 (D' = 1.00, R^2 = 0.89; Table 6) and significantly associated with adult asthma. Strong LD between T1 and T2 (D' = 0.98, R^2 = 0.73) was also found in an association study of a Japanese population (Hirota et al., 2006). In the present study, multi-locus haplotype analysis showed that the *ADAM33* haplotype profile was significantly different between patients and controls. Our results suggested that the Hap3 (CAC) and Hap4 (CAG) haplotypes are risk factors for asthma, whereas the Hap2 (TGC) haplotype may be a protective factor against the disease (Table 7).

The influence of *CD14* on the congenital immune response is determined based on gene expression levels (Simpson et al., 2006). As the promoter is well known to play an important role in regulating gene expression, studies examining *CD14* gene polymorphisms focus primarily on the promoter region. Polymorphisms in the *CD14* promoter region have been associated with asthma and hypersensitivity disease in studies of American (Baldini et al., 1999), Czech, Latin American (Choudhry et al., 2005), Japanese (Inoue et al., 2007), and Korean (Hong et al., 2007) populations. In a study of Chinese Han children, the promoter polymorphism -159C/T in *CD14* was associated with congenital allergies (Leung et al., 2003). Notably, the T allele of this SNP is the major allele in Asian populations (Tan et al., 2006; Hong et al., 2007), whereas the C allele is the major allele in American, European, and Australian populations (Jackola et al., 2006; Kedda et al., 2005). In our study, the T allele was the major allele, accounting for 57.1% and 56.0% in the asthma group and control group, respectively. This is consistent with previous results determined for Asian populations. Our logistic regression analysis showed that the polymorphisms of -159T/C, -1145G/A, and -1359G/T were not associated with asthma, T-IgE, or S-ECP before adjustment and after adjusting for confounding factors. A 10-year study of German neonates found that the -159C/T polymorphism was not associated with asthma, atopic dermatitis, allergic rhinitis, or the expression standard of IgE, suggesting that this SNP does not contribute to congenital allergic reactions in German children (Sengler et al., 2003). Similar findings have been reported for Polish Caucasian children (Heinzmann et al., 2003). In this study, we found no association between *CD14* haplotypes with asthma, T-IgE, and S-ECP. A study of T-IgE and sCD14 in 390 children suf-

fering from respiratory disease showed that the -1359T/-1145A/-159C haplotype had the highest T-IgE standard and the lowest sCD14 standard (Vercelli et al., 2001). Similar results were reported in another study of 301 school children of Chinese Han ethnicity (Tan et al., 2006).

The -896A/G polymorphism in *TLR4* may lead to changes in the cellular content of TLR4. Although there have been several reports regarding the association between *TLR4* polymorphisms and atopic diseases such as asthma, these results are often conflicting. Some studies showed that the polymorphisms Asp299Gly and Thr399Ile in *TLR4* were primarily found in the countries of both Europe and Africa, whereas the haplotype Asp299Gly/Thr399Ile was mainly found in European countries (Ferwerda et al., 2007). In the present study, we observed no Asp299Gly and Thr399lle polymorphisms in our sample, which is consistent with previous reports on Japanese, Korean, and Chinese Han populations (Hang et al., 2004; Noguchi et al., 2004; Kim et al., 2008). These findings suggest that the Asp299Gly and Thr399lle polymorphisms are very rare in Asian populations.

In summary, we showed that the T1, T2, and V4 polymorphisms in *ADAM33* may contribute to the susceptibility to asthma. A potential mechanism of the association between T1 and V4 and asthma may be due to a decrease in FEV1 levels, which may lead to increased asthma susceptibility. Specific haplotypes of *ADAM33* may contribute to higher asthma susceptibility in the Chinese Uygur population.

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Conflicts of Interest

The authors declare no conflict of interest.

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