ADAM33 gene polymorphisms identified to be associated with asthma in a Chinese Li population

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Abstract. A disintegrin and metalloprotease 33 (*ADAM33*) is an asthma susceptibility gene that has been proven to be present in certain human populations. The Li population is a minority ethnic group, most of whom maintain a distinctive lifestyle on Hainan Island in southern China. To the best of our knowledge, no previous study has established whether *ADAM33* polymorphisms are associated with asthma in the Li population. Therefore, the *ADAM33* polymorphisms in a Li population were investigated in the present study. A total of 150 asthma patients and 100 healthy subjects were enrolled in the present study, and their DNA samples were evaluated to analyze eight single-nucleotide polymorphisms (SNPs) on the *ADAM33* gene. Asthma patients were subcategorized into low and high severity groups, and their SNP data were compared with the data of the control subjects. Single-marker and haplotype association was analyzed to demonstrate the association between *ADAM33* SNPs and asthma using multiple genetic statistic tests. The results indicated significant differences in allele frequencies at the SNPs rs44707/T2 (P=0.008), rs2787094/V4 (P=0.028) and rs2280089/T+1 (P=0.021) between asthma patients and control subjects. The SNP rs44707/T2 was also found to be associated with the high severity group (P=0.024), although SNPs rs2787094/V4 were associated with the low severity group (P=0.019). Two haplotypes, GGAGAGT and GAAGGGT, were significantly associated with asthma (P=0.003 and 0.008, respectively). To the best of our knowledge, this is the first time that SNP rs2280089/T+1 has been reported to be associated with asthma in an Asian population. These data confirm that *ADAM33* polymorphisms are associated with asthma susceptibility in the Li population and confirm the uniqueness of the Li minority population within China.

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Introduction

Asthma is a chronic inflammatory disease of the airway. More than 100 million people across the globe suffer from this disorder (Global strategy for asthma management and prevention; http://www.ginasthma.org). Airway inflammation, bronchial hyperresponsiveness (BHR), and reversible airflow obstruction are the primary characteristics of asthma. Among these, airway inflammation is most closely associated with the clinical symptoms. Currently, genetic and environmental factors have been found to be involved in the generation of asthma pathologies. Thus, the potential contribution of genetic factors is considered to be an interesting area of clinical and basic research.

The A disintegrin and metalloprotease 33 (*ADAM33*) gene is an asthma susceptibility gene, which was first reported by Van Eerdewegh *et al* (1). The *ADAM33* gene is mapped to the short arm of chromosome 20p13 in the human genome, and is predominantly expressed in airway smooth-muscle cells and lung fibroblast cells, but not in epithelial cells, T cells or inflammatory leukocytes (2-6). ADAM proteins are involved in cell-cell and cell-matrix interactions (5), cell migration (2,3), cell-cell adhesion and signal transduction (4). The following are characteristic of *ADAM33*: Protease activity, a domain structure composed of a signal sequence, a prodomain, a metalloprotease domain, a transmembrane domain, a cysteine-rich domain, a disintegrin domain and a cytoplasmic domain (6). At present, >100 single-nucleotide polymorphisms (SNPs) of the *ADAM33* gene have been reported to be associated with asthma or BHR (7-18). However, the data that demonstrate the association of *ADAM33* SNPs with asthma were obtained from individuals living in mainland areas, who were predominantly Caucasian and Asian (10,11,19-21), while there is little data on *ADAM33* SNPs and asthma for ethnic minorities, particularly those living in isolated islands who seldom intermix with other ethnic groups. The Li population

is a unique ethnic minority whose members live only on the Hainan Island in southern China. Various lines of evidence have shown there to be various specific genetic variations in the Li population, differentiating it from the Han population, even from the Han population from the same island (22-25). Therefore, in the present study, the prevalence and types of *ADAM33* polymorphisms in the asthmatic individuals of the Li population were determined and compared with those in the control subjects.

Materials and methods

Study participants. The present study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all individuals and the protocols in the study were approved by the College's Ethics Committee (approval ID. HNMCE10011-9). Asthma was diagnosed according to the Global Initiative for Asthma (GINA) criteria (Global strategy for asthma management and prevention; http://www.ginasthma.org). The Li population study subjects were recruited between March 2008 and October 2013 from The People's Hospital of Sanya City (Sanya, China). A total of 150 patients with asthma (92 men and 58 women; mean age, 22.37 years) and 100 healthy controls (59 men and 41 women; mean age, 21.71 years) were enrolled. Subjects with asthma exhibited a mean forced expiratory volume in 1 sec (FEV1) of 1.79 l (64.58% of predicted), a mean forced vital capacity (FVC) of 2.37 l (79.82% of predicted), and a mean FEV1/FVC of 79.64%. The mean duration of asthma was 9.27 years. No significant difference between asthma patients and the normal controls was noted, except for lung function parameters. Pregnant and lactating women were excluded from the current study.

Extraction of the DNA samples. Clinical data and peripheral blood samples (5 ml per subject) were obtained from 150 unrelated asthma patients from the Li population and 100 unrelated healthy individuals who had no history of asthma. The genomic DNA samples were extracted from the peripheral blood of the subjects using a Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and the DNA samples were stored at -80˚C.

Polymerase chain reaction (PCR) and sequencing analysis. PCR-restriction fragment length polymorphism was performed to analyze the *ADAM33* SNPs using eight pairs of primers (Table I) as reported previously (26,27). PCR reactions were performed in a 25 μl system, as described previously (27). The PCR was run for 40 cycles of denaturation at 94˚C for 30 sec, annealing at the optimal melting temperature (60-65˚C) for 30 sec and extension at 72˚C for 1 min on a T-Gradient ThermoBlock (Biometra GmbH, Göttingen, Germany). In addition, sequencing of the resultant PCR products was performed by a commercial biotech company (Takara Biotechnology Co., Ltd., Dalian, China) to confirm the exact alleles.

Statistical analysis. The software SNPAnalyzer (version 2.0; Istech, Kyungkido, Korea) was used to calculate Lewontin's D' value, and analyze the linkage disequilibrium (LD) and eight *ADAM33* SNPs. Hardy-Weinberg equilibrium was confirmed via the exact distribution of allele frequencies using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference. The online software Haploview (http://www.broad.mit.edu/mpg/haploview) was used to construct the haplotype block. In addition, haplotype association analysis was conducted in two steps, as previously described (7,27). In order to determine whether *ADAM33* polymorphisms are associated with the severity of asthma, the asthma patients were further sub-categorized into high and low severity groups. The high severity group included subjects suffering from moderate to severe persistent asthma, whereas the low severity group consisted of subjects with intermittent and mild persistent asthma. The participants were diagnosed according to the GINA criteria (http://www.ginasthma.org).

Results

Demographic characteristics of study subjects. A total of 150 patients suffering from asthma (92 men and 58 women; mean age, 22.37 years) and 100 healthy control subjects (59 men and 41 women; mean age, 21.71 years) were enrolled into the current study and the SNPs of their *ADAM33* genes were analyzed. The major demographic characteristics are presented in Table II. The mean duration of asthma was 9.27 years. The demographic and lung function data indicated that the asthma patients had significantly lower lung function parameters than the healthy control subjects. The mean FEV1 and mean FVC in asthma patients were 1.79 l (64.58% of predicted) and 2.37 l (79.82% of predicted), respectively. To determine whether *ADAM33* gene polymorphisms are associated with the severity of asthma, the asthmatic patients were divided into a high severity group and low severity group. As shown in Table III, the age of the asthma patients in the high severity group was significantly older than that in the low severity group ($P<0.0001$), and the periods during which the patients had suffered from asthma in the high severity group was longer than in the low severity group ($P=0.006$). In addition, the mean FEV1 in the high severity group was significantly lower than that in the low severity group ($P <0.0001$).

Genotype frequencies. Eight *ADAM33* SNPs, including rs44707/T2, rs511898/F+1, rs528557/S2, rs612709/Q-1, rs2280089/T+1, rs2280091/T1, rs2787094/V4 and rs3918396/S1 have been reported to be associated with asthma in many of the world's mainland areas, and the majority of studies were performed on Caucasians and Asian individuals (10,11,19-21). In the present study, genotyping of these eight *ADAM33* SNPs was performed to evaluate the association with asthma in a Chinese Li population. These data indicated that all eight *ADAM33* SNPs were present and distributed in Hardy-Weinberg equilibrium in the Li population. The genotype frequencies of each SNP are presented in Table IV.

ADAM33 polymorphisms and association with asthma. Among the eight SNPs observed in the current study, the data shown in Table V demonstrated that only three SNPs, including rs44707/T2 ($P=0.008$), rs2280089/T+1 ($P=0.021$) and rs2787094/V4 ($P=0.028$) were significantly different in the Li asthma patients when compared with the control subjects. All of these three *ADAM33* SNPs were found to be associated with asthma in the dominant model. Notably, the

Table I. General information and oligonucleotide primers used for the amplification of *ADAM33* SNPs.

rs number	SNP	Alleles	Primers (5'-3')	Length of enzyme and digested area (bp)
rs44707	T2	G >A	F: TTCTCAGGGTCTGGGAGAAA R: GCCAACCTCCTGGACTCTTA	*HpyCH4III* A:198+112, G:310
rs511898	F+1	C >T	F: GTATCTATAGCCCTCCAAATCAGAAGAGCC R: GGACCCTGAGTGGAAGCTG	*BsmBI* C:208+192, T:400
rs528557	S2	G >C	F: AGAGCTCTGAGGAGGGGAACCG R: GCAGACCATGACACCTTCCTGCTG	*FseI*, C:148+156 G:304
rs612709	Q-1	G >A	F: GGATTCAAACGGCAAGGAG R: GTTCACCTAGATGGCCAGGA	*BtsCI*, A:20+138 G:158
rs2280089	T+1	G >A	F: CTGAGCCCAGAAACCTGATT R: AGAAGGGAAGGGCTCATGC	*HpyAV,* A:284+28, G:312
rs2280091	T1	A >G	F: ACTCAAGGTGACTGGGTGCT R: GAGGGCATGAGGCTCACTTG	*NcoI*, A:140+260, G:400
rs2787094	V4	C >G	F: CTCAGGAACCACCTAGGGGAGAAG R: CAAAGGTCACACAGCCCCTGACCT	*PstI*, G:168+206, C:374
rs3918396	S1	G >A	F: TGTGCAGGCTGAAAGTATGC R: AGAGCTCTGAGGAGGGGAAC	*HinfI*, G:132+172, A:304

ADAM33, A disintegrin and metalloprotease 33; SNP, single nucleotide polymorphism; F, forward; R, reverse.

Table II. Demographic characteristics of the asthmatic patients (n=150) and control subjects (n=100).

Characteristic	Case	Control	P-value
Age (years)	22.37±19.72	21.71±20.64	0.482[a]
Gender (M/F)	92/58	59/41	0.819[b]
Duration of asthma (years)	9.27±3.28		
Pack year of smoking	31.53±6.22	29.57±5.81	0.052[a]
FVC (% of predicted)	79.82±15.66	94.81±8.42	<0.0001[a]
FEV1 (% of predicted)	64.58±13.85	92.77±7.52	<0.0001[a]

Values for age, smoking status, FVC and FEV1 are expressed as means ± standard deviation. The value for gender is expressed as the ratio between males and females. P-values obtained by [a]t-tests and [b]Fisher's exact test. M, male; F, female; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 sec.

Table III. Demographic characteristics of the asthmatic patients in high (n=62) and low (n=88) severity groups.

Characteristic	High severity	Low severity	P-value
Age (years)	31. 27±18.83	20.61±19.21	<0.0001[a]
Gender (M/F)	38/24	50/38	0.361[b]
Duration of asthma (years)	11.57±9.05	7.82±5.99	0.006[a]
FVC (% of predicted)	78.94±17.22	82.41±20.79	0.091[a]
FEV1 (% of predicted)	57.41±14.91	77.35±16.33	<0.0001[a]

Values for age, duration of asthma FVC and FEV1 are expressed as mean ± standard deviation. The value for gender is expressed as the ratio between males and females. P-values obtained by [a]t-tests and [b]Fisher's exact test. M, male; F, female; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 sec.

association of rs2280089/T+1 with asthma was only observed in European and Latin American individuals (28). To the best of our knowledge, no previous study has demonstrated such an association in Asian individuals (28) and this is the first time

Table IV. Genotype frequencies of *ADAM33* SNPs in the Li population.

		Genotype [Case (n = 150)/control (n=100)]		
SNP ID	SNP	Homozygous (wild-type)	Heterozygous	Homozygous (variant)
rs44707	T2	GG (82/71)	GA (58/21)	AA (10/8)
rs511898	F+1	CC (51/46)	CT (81/42)	TT (18/12)
rs528557	S2	GG (48/39)	GC (78/41)	CC (24/20)
rs612709	Q-1	GG (66/54)	GA (74/38)	AA (10/8)
rs2280089	T+1	GG (80/75)	GA (59/19)	AA (11/6)
rs2280091	T1	AA (121/87)	AG (28/13)	GG (1/0)
rs2787094	V4	CC (83/73)	CG (60/19)	GG (8/7)
rs3918396	S1	GG (148/98)	GA (2/1)	AA (0/1)

ADAM33, A disintegrin and metalloprotease 33; SNP, single nucleotide polymorphism.

Table V. *ADAM33* polymorphisms with asthma susceptibility in the Li population.

				χ^2 test	
SNP ID	SNP	Genetic model	Genotype	Odds ratio (95% confidence interval)	P-value
rs44707	T2	Dominant	GA + AA GG	1.97 (1.12-3.29)	0.008
rs2280089	T+1	Dominant	GA + AA GG	1.84 (1.14-3.21)	0.021
rs2787094	V4	Dominant	CG + GG CC	1.78 (1.09-2.97)	0.028

ADAM33, A disintegrin and metalloprotease 33; SNP, single nucleotide polymorphism.

that rs2280089/T+1 has been reported to be associated with asthma in an Asian population.

Haplotype association with asthma. Three *ADAM33* SNPs (rs44707/T2, rs2280089/T+1 and rs2787094/V4) that were statistically significant in the present study were selected to further analyze their haplotypes, which was performed using Haploview 4.2 software, as previously reported (26). The eight haplotypes with frequencies >2.0% were identified to be suitable for further analysis. Table VI demonstrates the frequency of GGAGAGT and GAAGGGT haplotypes to be significantly higher in the asthmatic patients than in the control subjects (P=0.003 and P=0.008, respectively). Furthermore, the SNPs rs44707/T2, rs2280089/T+1 and rs2787094/V4 demonstrated strong *D*' values in pair-wise LD analysis (D'=0.9012 between rs44707/T2 and rs2280089/T+1; D'=0.8931 between rs2280089/T+1 and rs2787094/V4; Fig. 1), confirming the validity of the present haplotype analysis.

Association of SNPs in high and low severity patients. In the present study, the asthma patients were sub-grouped into high and low severity groups, and the allele and genotype differences from healthy control subjects were analyzed. The clinical characteristics of the high and low severity groups are demonstrated in Table III. The data concerning allelic distribution analysis showed that allele frequencies differed significantly between the high severity group and the control group with respect to SNP rs44707/T2 (P=0.024; Table VII). In addition, the allele frequencies demonstrated significant differences between the low severity group and the control subjects for the SNP rs2787094/V4 (P=0.019; Table VII). However, no significant differences were noted between the high and low severity groups in the allele frequencies.

Discussion

Various previous studies have reported that *ADAM33* is a positively cloned gene for asthma (7-18). Thus, it is necessary to study the association of *ADAM33* and prevalence of asthma in different populations, particularly in those living in special geographical environments, such as China's Li population. There are 55 minority ethnic groups in China, including the Li population (~1.3 million), who reside primarily in the central and southwestern regions of Hainan Island in the South China Sea, of which they are the aboriginal inhabitants. The Li people have maintained much of their traditional lifestyle into the present day. Although certain members of the Li community have intermarried with other communities, such as the Han and Miao populations, most of the Li people have remained relatively isolated from other populations, meaning that they are likely to

Table VI. Frequency of each haplotype from the five-marker model in the Li population.

Haplotype	Haplotype frequency			P-value
	Case (n=150)	Control (n=100)	Combined (n=250)	
GGAGAGT	0.28	0.11	0.25	0.003
GAAGGGC	0.04	0.03	0.03	NS
GGAGGGC	0.03	0.01	0.02	NS
GGACGGT	0.12	0.09	0.10	NS
GGACGGC	0.04	0.05	0.04	NS
GAAGGGT	0.22	0.10	0.19	0.008
GAAGGAC	0.09	0.08	0.09	NS
GGGGAGT	0.05	0.04	0.04	NS

NS, not significant.

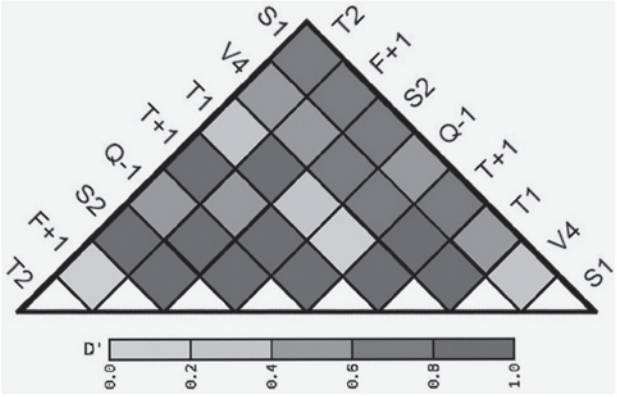

Figure 1. Pair-wise linkage disequilibrium analysis of the *ADAM33* gene in the Li population. S1, rs3918396; V4, rs2787094; T1, rs2280091; T+1, rs2280089; Q-1, rs612709; S2, rs528557; F+1, rs511898; T2, rs44707; ADAM33, A disintegrin and metalloprotease 33; D', Lewontin's D' value.

have retained any unique genetic traits. Thus, any genetic study of the Li people may help to promote personalized therapeutic strategies in Li patients. Currently, there is a dearth of data concerning the *ADAM33* polymorphisms in the Li population. Thus, the aim of the present study was to evaluate the variations in the *ADAM33* gene in the Li population. The present study focused on eight SNPs of the *ADAM33* gene that have been reported to be significantly closely associated with asthma or airway hyperresponsiveness in several populations (28). Gene segments covering these eight *ADAM33* gene SNPs were successfully amplified and the results indicated that three SNPs, rs44707/T2, rs2280089/T+1 and rs2787094/V4, were associated with asthma in a dominant model in a Li population.

ADAM33 is a polymorphic gene with >100 known SNPs (28). However, not all these SNPs are associated with asthma. A meta-analysis analyzed 14 SNPs of the *ADAM33* gene in 29 case-control studies in different populations, and the results showed significant associations with the rs2280091/T1, rs2787094/V4, rs511898/F+1 and rs2280089/T+1 polymorphisms in the overall population (28). However, positive results

Table VII. *ADAM33* polymorphisms with asthma susceptibility in high and low severity patients in the Li population.

SNP ID	SNP	Genotype	High severity/ control P-value	Low severity/ control P-value
rs44707	T2	GA + AA GG	0.024	NS
rs2280089	T+1	GA + AA GG	NS	NS
rs2787094	V4	CG + GG CC	NS	0.019

ADAM33, A disintegrin and metalloprotease 33; NS, not significant.

were only found for the rs2280091/T1, rs2787094/V4, rs511898/F+1 and rs511898/T2 polymorphisms, and only in Asians, not European or Latin American individuals (28). In the current study, a specific phenomenon regarding *ADAM33* SNPs involving rs2280089/T+1 was observed, which was also observed in European and Latin American individuals, but never in Asian individuals (28). To the best of our knowledge, this is the first study to report that rs2280089/T+1 is associated with asthma in an Asian population. Although the underlying mechanism behind the *ADAM33* SNP remains unknown, this finding indicates that the Li population is a particularly unique minority population in China and the world.

In the present study, the data also demonstrated *ADAM33* SNPs to be significantly closely associated with the severity of asthma in the Li population. When compared with the control subjects, significant associations with high severity asthma were found in SNP rs44707/T2 (P=0.024), whereas a significant association with low severity asthma patients was observed in SNP rs2787094/V4 (P=0.019). Therefore, the results are consistent with previous results where asthma patients demonstrated significant decreases in lung function over time (29).

Haplotypes contain specific information regarding potentially unique, unobserved predisposing variants in specific regions (28,30). In the present study, two common haplotypes, including GGAGAGT and GAAGGGT, were found to be asthma-predisposing variants in the Li population. Haplotype data obtained from other unrelated subjects showed there to be a significant association between specific *ADAM33* haplotypes and asthma in Chinese Han, Chinese Uygur, Japanese and German populations, and with BHR in a Korean population (28). Furthermore, family-based studies of German asthmatic patients confirmed the association of the *ADAM33* haplotype with asthma (17). However, a variety of haplotype analyses in asthma subjects have already been identified in different populations. The previous results suggested that a risk haplotype in one population may be a protective haplotype in another (28). However, in the present study, no significant differences were identified in the distribution of haplotypes between the high and low severity asthma groups. This may be due to the relatively small sizes of the asthmatic patient populations. In a previous study, >200 asthmatic subjects were observed over a period of >20 years, and the resultant

data showed the *ADAM33* gene SNPs to be associated with a decline in FEV1, indicating that the function of the *ADAM33* gene may be involved in all pathologic steps, including asthma prevalence, severity, airway hyperresponsiveness, progression and prognosis (9). Various lines of evidence in previous studies indicated that overexpression of the *ADAM33* gene (mRNA and protein) was seen in various types of cells in asthma patients, including airway smooth muscle cells, fibroblasts, mesenchymal cells and endothelial cells (2-6). Furthermore, data from previous studies have demonstrated that the expression of the *ADAM33* gene may regulate the expression of transforming growth factor-β, which is a major cytokine expressed and excreted by bronchial macrophages, epithelial cells and mesenchymal cells for airway remodeling (31). A potential mechanism by which *ADAM33* affects asthma may be associated with the regulation of airway inflammation, hyperresponsiveness and remodeling processes; however, the exact molecular mechanism requires further investigation.

In conclusion, these data demonstrated a positive association between *ADAM33* gene SNPs and asthma prevalence in a Li minority population in southern China. These data strengthen the evidence from previous investigations and studies of other populations elsewhere in the world. Currently, the exact functions of the *ADAM33* gene in the progress of asthma pathogenesis remain unknown. Further investigations are required to determine the exact mechanism and the exact role of the *ADAM33* gene in asthma.

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