



## Original article

## Association of ADAM33 gene polymorphisms with asthma in Mongolian and Han groups in Inner Mongolia

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## ABSTRACT

Polymorphisms in the gene encoding for A disintegrin and metalloprotease 33 (ADAM33) are closely associated with the risk of bronchial asthma attacks in different populations. We collected blood samples from 248 asthma patients – 130 of the Han ethnic group and 118 of the Mongolian ethnic group – living in the Inner Mongolia region of China, and analyzed the single nuclear polymorphisms (SNPs) of the T1, T2 and V4 loci of the ADAM33 gene using PCR-RFLP (restriction fragment length polymorphism). In addition, we also tested 256 healthy controls (134 and 122 from the Han and Mongolian ethnic groups respectively) for the same SNPs. Three genotypes of the T1, T2 and V4 loci were predominantly detected: while polymorphisms in the T1 locus were significantly associated with asthma risk in both Mongolian and Han ethnicities ( $P < 0.05$ ,  $*P < 0.05$ ), that in the V4 locus were relevant only in the Mongolian patients ( $P < 0.05$ ,  $*P > 0.05$ ). In contrast, polymorphisms in the T2 locus showed no significant association with asthma risk in either ethnic group ( $P > 0.05$ ,  $*P > 0.05$ ).

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## 1. Introduction

Bronchial asthma is a common chronic airway disease, and its clinical control rate is at present lower than that recommended in the GINA guide, making this a serious public health issue (Asthma group of respiratory disease branch of Chinese Medical Association, 2008). It is a chronic inflammatory condition mediated via multiple cell types and molecules, resulting in airway hyper-responsiveness that causes an extensive, volatile and reversible limitation of airway function. Bronchial asthma has a complex pathogenesis and is dependent on immunological, genetic and environmental factors, and recent studies indicate a hereditary basis. Therefore, it is essential to identify the genes that determine the susceptibility to bronchial asthma. Variations in the A Disintegrin And Metalloprotease 33 (ADAM33) gene have recently been correlated to increased risk of asthma attacks. The ADAM33 gene has 179 single nucleotide polymorphism (SNP) loci, of which the

T1, T2 and V4 have been the main focus of research. No reports have so far been published on the association between the T1, T2 and V4 SNPs and asthma risk in the ethnic Mongolian groups of the Inner Mongolia Autonomous Region. We used the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique to map the ADAM33 SNPs among the healthy and bronchial asthma patients of Han and Mongolian ethnicities of Inner Mongolia to further explore the genetic basis of bronchial asthma.

## 2. Materials and methods

**Case collection** Bronchial asthma inpatients or outpatients of the Mongolian and Han ethnicities from the affiliated hospital of Inner Mongolia Medical University were chosen as the objects of study along with a group of Mongolian and Han people who were considered healthy according to the results of physical checkups performed during the corresponding period. All selected asthma patients had lived in the Inner Mongolia region for three or more generations, and did not share any genetic relations, had no history of mixed marriage, no chronic pulmonary disease, no allergic dermatitis or other history of allergic diseases, no chronic underlying disease such as hypertension and diabetes, and no history of cancer, autoimmune disease or familial hereditary disease. Of the 130 asthma patients from the Han ethnic group, 72 were males and 58 were females, ranging from 21 to 85 years of age (average

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age:  $44 \pm 13.4$ ). Of the 118 Mongolian asthma patients, there were 58 males and 60 females, ranging from 17 to 76 years of age (average age:  $37 \pm 11.5$ ). All cases met the diagnostic criteria for asthma revised by the asthmology team of the Respiratory Society of the Chinese Medical Association (2008). The normal control group included 134 healthy subjects of the Han ethnic group and 122 healthy subjects of the Mongolian ethnic group. There were no significant age, gender composition or comparative differences in the research subjects in this study ( $P < 0.05$ ). All patients included in this study have signed informed consents.

**Genome DNA extraction** 2 mL peripheral venous blood was taken from each subject and sent for anticoagulation using EDTA (INSEPACK, ST520EK). A whole blood genome DNA extraction kit (TIANGEN, DP348-02) was used to extract DNA, which was then stored at a temperature of  $-80^\circ\text{C}$ .

**Primer design** According to the gene sequence provided by GenBank, primers were designed using Primer Premier 5 software. Primer sequences were synthesized by Shanghai Biological Engineering Co., Ltd. For details, see Table 1. Classification was performed based on SNaPshot quality control over double-blind samples and negative control for the classification results.

**PCR amplification** The PCR reaction system totaled 20  $\mu\text{L}$ , which included: genome DNA 1  $\mu\text{L}$ , Extaq Mix 10  $\mu\text{L}$ , upstream primer 1  $\mu\text{L}$ , downstream primer 1  $\mu\text{L}$ , and ddH<sub>2</sub>O 7  $\mu\text{L}$ . PCR reaction conditions: 5 min pre-denaturation under  $95^\circ\text{C}$ , 20 s denaturation under  $95^\circ\text{C}$ , 20 s annealing under  $60\text{--}68^\circ\text{C}$ , 30 s extension under  $72^\circ\text{C}$ , with 35 cycles in total, 5 min terminal extension under  $72^\circ\text{C}$ . PCR product was tested using 2% agarose gel electrophoresis, and DNA sequencing was performed.

**RFLP detection** The NcoI, HpyCH4III and PstI restriction enzymes were used for digestion detection of the T1, T2 and V4 loci. Enzyme digestion system: the system totaled 20  $\mu\text{L}$ , containing amplified PCR reaction product 10  $\mu\text{L}$ , restriction enzyme 0.5  $\mu\text{L}$ , ddH<sub>2</sub>O 7.5  $\mu\text{L}$ , and  $10 \times$  NE Buffer fluid 2  $\mu\text{L}$ . It was placed at a temperature of  $37^\circ\text{C}$  for one hour and  $65^\circ\text{C}$  for 20 min. 3% sepharose gel was used for electrophoresis of the enzyme-digested product for 30 min, with voltage maintained at 120 V. After gel imaging, Quantity One 1-D software was used to perform image analysis.

**Statistical analysis** SPSS 18.0 software was used for statistical analysis. Measurement data are expressed in the form of  $\bar{x} \pm s$ , and the two measurement data groups were compared using the *t* test. Enumeration data groups were compared using the  $\chi^2$  test. Gene frequency was calculated based on the gene counting method. The  $\chi^2$  test was conducted to analyze whether genotype distribution was consistent with the Hardy-Weinberg law of genetic equilibrium. The  $\chi^2$  test was also adopted to compare inter-group genotypes and allele frequencies. Moreover, OR values and 95% CI were calculated. AP value of less than 0.05 represented a statistically significant difference.

### 3. Results

#### 3.1. Genotype test results of T1, T2 and V4 loci

**T1 locus: A/G mutation type** For the AA genotype, PCR products could be completely digested by NcoI enzymes, and the enzyme-

digested product fragments were 144 bp and 256 bp long. For the AG genotype, PCR products could be partially digested by NcoI enzymes, resulting in three fragments with lengths of 144 bp, 256 bp and 400 bp. For the GG genotype, PCR products could not be digested by NcoI enzymes, and the enzyme-digested product was 400 bp long (Fig. 1A).

**T2 locus: G/A mutation type** For the AA genotype, PCR products could be completely digested by HpyCH4III enzymes, and the lengths of the enzyme-digested product fragments were 200 bp and 110 bp. For the AG genotype, PCR products could be partially enzyme-digested, generating three fragments with lengths of 110 bp, 200 bp and 310 bp. For the GG genotype, PCR products could not be digested by HpyCH4III enzymes (Taal), and the length of the enzyme-digested product was 310 bp (Fig. 1B).

**V4 locus: C/G mutation type** For the GG genotype, PCR products could be completely digested by PstI enzymes, and the enzyme-digested product fragments were 172 bp and 202 bp long. For the GC genotype, PCR products could be partially digested by enzymes, resulting in three segments with lengths of 172 bp, 202 bp and 374 bp. For the CC genotype, PCR products could not be digested by PstI enzymes, and the length of the enzyme-digested product was 374 bp (Fig. 1C).

#### Distribution of T1, T2 and V4 locus genotypes and allele frequencies

- Comparison of T1, T2 and V4 locus genotypes and allele frequency distribution between the healthy Mongolian group and the healthy Han group There were 134 cases in the healthy Han ethnic group and 122 cases in the healthy Mongolian ethnic group. The three ADAM33 genotypes (T1, T2 and V4) were detected in all cases. Genotypes and allele frequencies of the loci were compared using the chi-squared test. Differences between the two groups were not statistically significant ( $P > 0.05$ ). For details (Table 2).
- Comparison of T1, T2 and V4 locus genotypes and allele frequency distribution between the Han asthma group and the healthy Han group There were 134 cases in the healthy Han group and 130 cases in the Han asthma group. The three

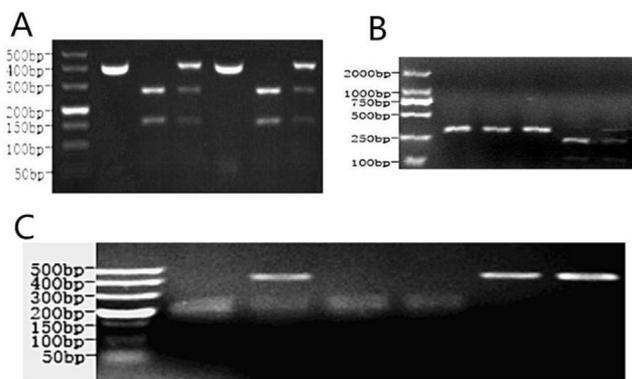


Fig. 1. Genotype test results of T1, T2 and V4 loci.

Table 1  
Primer sequences of ADAM33 PCR-RFLP.

SNP	Primer sequence	PCR product (b.p.)	Annealing temperature	Restriction enzyme
T1	Forward: 5'-ACTCAAGGTGACTGGGTGCT-3'	400	68 °C	NcoI
rs2280091	Reverse: 5'-GAGGGCATGAGGCTCACTTG-3'			
T2	Forward: 5'-TTCTCAGGTCTGGGAGAAA-3'	310	60 °C	HpyCH4III
rs2280090	Reverse: 5'-GCCAACCTCTGGACTCTTA-3'			
V4	Forward: 5'-ACACACAGAATGGGGAGAG-3'	374	66 °C	PstI
rs2787094	Reverse: 5'-CCAGAAGCAAAGTACACA-3'			

**Table 2**

Comparison of T1, T2 and V4 locus genotypes and allele frequency distribution between the healthy Mongolian group and the healthy Han group.

SNP	Genotypes and alleles	Healthy Han group (n = 134)	Healthy Mongolian (n = 122)	$\chi^2$	P
ADAM33 T1	AA	94 (70.15)	88 (72.13)	0.122	0.727
	AG	37 (27.61)	31 (25.41)	0.159	0.690
	GG	3 (2.24)	3 (2.46)	0.000	1.000
	A	225 (83.96)	207 (84.84)		
	G	43 (16.04)	37 (15.16)	0.075	0.784
ADAM33 T2	GG	99 (73.88)	94 (77.05)	0.346	0.557
	GA	32 (23.88)	22 (18.03)	1.312	0.252
	AA	3 (2.24)	6 (4.92)	0.677	0.411
	G	230 (85.82)	210 (86.07)		
	A	38 (14.18)	34 (13.93)	0.006	0.937
ADAM33 V4	CC	64 (47.76)	63 (51.64)	0.384	0.535
	GC	37 (27.61)	31 (25.41)	0.159	0.690
	GG	33 (24.63)	28 (22.95)	0.099	0.753
	C	165 (61.57)	157 (64.34)		
	G	103 (38.43)	87 (35.66)	0.422	0.516

ADAM33 genotypes (T1, T2 and V4) were detected in all cases. The chi-squared test was conducted to compare the genotypes and allele frequencies of the T1 locus between the two groups. According to the results, there was a statistically significant difference between the two groups in terms of the AA and AG genotypes ( $P < 0.05$ ), and the OR values (95% CI) for the two groups were 2.089 (1.159–3.765) and 0.449 (0.242–0.831), respectively. The OR value (95% CI) of allele G was 0.557 (0.329–0.942). The T2 and V4 locus genotypes and allele frequencies of the two groups were also compared. The difference was of no statistical significance ( $P > 0.05$ ). For details (Table 3).

- c. Comparison of T1, T2 and V4 locus genotype and allele frequency distribution between the Mongolian asthma group and the healthy Mongolian group There were 122 cases in the healthy Mongolian group and 118 cases in the Mongolian asthma group. The three ADAM33 genotypes (T1, T2 and V4) were detected in all cases. The chi-squared test was conducted to compare genotypes and allele frequencies of the T1 locus between the two groups. According to the results, there was a statistically significant difference between the two groups in terms of AA and AG genotypes ( $P < 0.05$ ), and OR values (95% CI) were 2.295 (1.200–4.391) and 0.395 (0.198–0.789), respectively. The OR value (95% CI) of allele G was 0.518 (0.291–0.922). The V4 locus genotypes and allele frequencies of the two groups were compared. Results indicate that there was a statistically significant difference between the groups in terms of GC and GG genotypes ( $P < 0.05$ ), and OR values (95%CI) were 0.363

(0.179–0.736) and 2.555 (1.463–4.462), respectively. The OR value (95%CI) of allele G was 1.715 (1.190–2.473). A comparison was also made between the T2 locus genotypes and allele frequencies between the two groups. There was no statistically significant difference between the two groups ( $P > 0.05$ ). For details (Table 4).

#### 4. Discussion

ADAM33, a member of the ADAM family, is a 14 kb long gene located on the short arm (20p13) of chromosome 20, and consists of 22 exons and 21 introns. It encodes an 813 amino acid-long matrix metalloproteinase, and has a long 3' untranslated (UTR) region containing 7 polymorphic sites. Gene polymorphisms are often manifested as different protein isoforms via alternate mRNA splicing and translation. ADAM33 transcription shows tissue specificity, with predominant expression in pulmonary smooth muscle cells and fibroblasts, and lower levels in the airway epithelial cells, T cells and other immune cells. Changes in ADAM33 expression can therefore lead to functional changes in the airway smooth muscle cells and fibroblasts. It has also been correlated with cellular repair after damage to airway epithelial cells, indicating a potential role in small airway remodeling, hyper-responsiveness and inflammation. In addition, studies show a close association of ADAM33 with bronchial asthma.

Polymorphisms in the human ADAM33 gene are seen across races, ethnicities, and geographical regions. Van Eerdewegh et al were the first to discover a strong correlation between 14 SNPs of the ADAM33 gene and asthma prevalence in the Caucasian

**Table 3**

Comparison of T1, T2 and V4 locus genotypes and allele frequency distribution between the Han asthma group and the healthy Han group.

SNP	Genotypes and alleles	Han asthma group (n = 130)	Healthy Han group (n = 134)	P	OR	95%CI
ADAM33 T1	AA	108 (83.08)	94 (70.15)	0.013	2.089	1.159–3.765
	AG	19 (14.61)	37 (27.61)	0.010	0.449	0.242–0.831
	GG	3 (2.31)	3 (2.24)	1.000	1.031	0.204–5.206
	A	235 (90.38)	225 (83.96)			
	G	25 (9.62)	43 (16.04)	0.027	0.557	0.329–0.942
ADAM33 T2	GG	105 (80.77)	99 (73.88)	0.182	1.485	0.830–2.657
	GA	24 (18.46)	32 (23.88)	0.282	0.722	0.398–1.308
	AA	1 (0.77)	3 (2.24)	0.636	0.339	0.035–3.297
	G	234 (90.00)	230 (85.82)			
	A	26 (10.00)	38 (14.18)	0.026	0.532	0.303–0.934
ADAM33 V4	CC	70 (53.85)	64 (47.76)	0.323	1.276	0.787–2.069
	GC	36 (27.69)	37 (27.61)	0.988	1.004	0.585–1.722
	GG	24 (18.46)	33 (24.63)	0.224	0.693	0.383–1.253
	C	176 (67.69)	165 (61.57)			
	G	84 (32.31)	103 (38.43)	0.141	0.765	0.535–1.094

**Table 4**

Comparison of T1, T2 and V4 locus genotype and allele frequency distribution between the Mongolian asthma group and the healthy Mongolian group.

SNP	Genotypes and alleles	Mongolian asthma group (n = 118)	Healthy Mongolian group (n = 122)	P	OR	95%CI
ADAM33 T1	AA	101 (85.59)	88 (72.13)	0.011	2.295	1.200–4.391
	AG	14 (11.86)	31 (25.41)	0.007	0.395	0.198–0.789
	GG	3 (2.54)	3 (2.46)	1.000	1.035	0.205–5.232
	A	216 (91.53)	207 (84.84)			
	G	20 (8.47)	37 (15.16)	0.024	0.518	0.291–0.922
ADAM33 T2	GG	89 (75.42)	94 (77.05)	0.767	0.914	0.504–1.657
	GA	24 (20.34)	22 (18.03)	0.650	1.161	0.610–2.208
	AA	5 (4.24)	6 (4.92)	0.801	0.855	0.254–2.882
	G	202 (85.60)	210 (86.07)			
	A	34 (14.40)	34 (13.93)	0.338	1.298	0.760–2.219
ADAM33 V4	CC	54 (42.37)	63 (51.64)	0.363	0.790	0.476–1.312
	GC	13 (11.02)	31 (25.41)	0.004	0.363	0.179–0.736
	GG	51 (43.22)	28 (22.95)	0.001	2.555	1.463–4.462
	C	121 (51.27)	157 (64.34)			
	G	115 (48.73)	87 (35.66)	0.004	1.715	1.190–2.473

population. Howard et al found that at least one of 8 SNPs (including T1, T2 and V4) in the 3' UTR of ADAM33 was correlated with asthma in the Dutch-American, Hispanic-American and African-American populations. Hirota et al discovered that the four loci (T1, T2, S2 and V-3) of ADAM33 were correlated to asthma in the Japanese population. Vergara et al found that the TT genotype of ST + 7 SNP was a risk factor for asthma in the Columbian population, while H4 (GCAGGG) was associated with familial asthma, and the V4 and T2 loci were correlated to blood IgE levels. However, Schedel et al did not find any connection between either the T1 or V4 loci and asthma or bronchial hyper-responsiveness in the German population. This finding was corroborated by Mona et al and Miyake et al who did not detect a clear correlation between the T1, T2 or V4 loci and asthma in the German and Japanese populations respectively. Qu et al found a significant correlation between pediatric asthma in northern China and the T1 and V4 loci out of a total of 6 SNPs. In a study involving Taiwanese asthma patients and 115 healthy individuals, Chiang found a significant difference in the frequency distribution of the AA, AG and GG genotypes, and of the A and G alleles between the two groups. However, Xiangyu et al failed to identify any connection between the T2 and V4 loci and asthma attacks in patients in the Shandong province. Furthermore, no correlation has been found between the T1 locus and asthma attacks among children in Guangzhou city. Studies on the Xinjiang Uyghur and Guangxin Zhuang ethnic minorities also show a correlation between asthma and polymorphisms in the T1, T2 and V4 loci. Taken together, although SNPs in these loci are highly prevalent in different regions and populations, their distribution and association with asthma risk varies greatly.

#### **T1 locus polymorphism is closely related to asthma attacks**

Both the T1 locus genotype and allele polymorphism were correlated ( $P < 0.05$ ) with the occurrence of asthma in the Mongolian and Han groups. The frequency of the AA and AG genotypes were significantly different between the asthmatic and healthy individuals in the Han population ( $P < 0.05$ ); the OR values (95%CI) were 2.089 (1.159–3.765) and 0.449 (0.242–0.831) respectively, and that of allele G was 0.557 (0.329–0.942). In the Mongolian population as well, a significant difference was seen in the frequencies of the AA and AG genotypes between the two groups ( $P < 0.05$ ), with OR values (95% CI) 2.295 (1.200–4.391) and 0.395 (0.198–0.789) respectively, and that of allele G 0.518 (0.291–0.922). These findings indicate a correlation between the AA genotype and higher asthma risk in both ethnic groups living in Inner Mongolia, with alleles G and A as the protective and susceptibility genes respectively. This conclusion is consistent with that of studies conducted in the Zhuang Autonomous region and the Taiwanese provinces, as

well as outside China. In conclusion, polymorphisms in the T1 locus are associated with asthma attacks.

**Locus T2 is not significantly correlated with asthma attacks** In contrast to the T1 locus, the T2 locus genotypes and allele polymorphisms were not significantly associated with asthma risk in either the Mongolian or Han populations ( $P > 0.05$ ). This contradicts the findings of Howard and Hirota, but is consistent with that of Mona and Xiangyu. Therefore, further research is required on more ethnic populations before concluding the role of the T2 in asthma risk.

**Polymorphism of the V4 locus varies among asthma patients of different ethnicities** For the V4 locus, the frequencies of the CC, GC and GG genotypes among the asthmatics were 54 (42.37%), 13 (11.02%) and 51 (43.22%), and that among the control group were 63 (51.64%), 31 (25.41%) and 28 (22.95%) respectively in the Mongolian population. A significant difference was seen in the prevalence of the GC and GG genotypes between the two groups ( $P < 0.05$ ), with respective OR values (95% CI) 0.363 (0.179–0.736) and 2.555 (1.463–4.462) and that of allele G 1.715 (1.190–2.473). Therefore, allele G is a likely asthma risk factor, while allele C is protective against asthma in the Mongolian population. However, no significant differences were seen among the healthy individuals and the asthmatics in the Han population. This indicates that polymorphisms in the ADAM33 V4 locus differ across ethnicities, and a mutation can either turn it to a susceptibility gene or prove insignificant. Further studies on larger cohorts are needed to verify the differences between the ethnic groups in the same region.

Although the Mongolian and Han groups in Inner Mongolia share the same region, asthma patients in the two populations differ in terms of susceptibility and protective genes. The correlation between polymorphism of the ADAM33 gene loci and asthma depends on both race and ethnicity, since this gene is highly polymorphic and mutations in different loci exert differential effects on gene function and expression. No studies have so far determined the exact genetic basis of asthma or the SNP loci that are functional in this disease. Further population studies are therefore needed to validate our findings and eliminate errors due to sample differences and experimental errors.

## **5. Conclusion**

In conclusion, China is a multi-ethnic country, and the prevalence rates of bronchial asthma for different ethnic groups and in different regions vary. Polymorphism of the ADAM33 gene T1 locus may have an effect on the asthma populations of the Mongolian and Han ethnic groups in China's Inner Mongolia, and

polymorphism of the V4 locus may only play a role in the asthma population of the Mongolian ethnic group. However, polymorphism of the ADAM33 gene T2 locus may be unrelated to the asthma population of the Mongolian and Han ethnic groups in Inner Mongolia.

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