

ADAM33 polymorphism: association with bronchial hyper-responsiveness in Korean asthmatics

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Summary

Background A disintegrin and metalloprotease 33 (ADAM33) is expressed in the lung by fibroblasts and bronchial smooth muscle cells. Given its structure and cellular provenance, ADAM33 may be associated with airway remodelling and bronchial hyper-responsiveness. Single nucleotide polymorphisms (SNPs) and haplotypes of the *ADAM33* gene have previously been associated with asthma susceptibility in the Caucasian population.

Objective and Methods To assess whether genetic variants of ADAM33 are related to asthma in a Korean population, we conducted an association study of the *ADAM33* gene with asthma susceptibility, bronchial hyper-reactivity and serum IgE in Korean asthmatics ($n = 326$) and normal controls ($n = 151$). Five of the 14 polymorphisms originally reported to be associated with asthma development (S1 G>A, T1 T>C, V-1 C>A, V1 T>A, V4 C>G) were genotyped using single base extension and electrophoresis. Haplotypes and their frequencies were inferred using the algorithm implemented by the software Arlequin. Allele frequencies of each SNP and haplotypes were compared between the patients and the normal controls using logistic regression analysis.

Results There was no significant difference in the distribution of SNPs and the six haplotypes between asthmatics and normal controls. All single SNPs and six haplotypes in ADAM33 were also analysed for the association with level of PC₂₀ using general linear models. The distribution of the T1 T>C SNP and one haplotype (ht4: GCGG) showed significant association with log-transformed PC₂₀ methacholine level in the asthma patients ($P = 0.03$ and 0.0007 , respectively, using a co-dominant model).

Conclusion Polymorphism of *ADAM33* may contribute to development of BHR in asthma.

Keywords *ADAM33* gene, asthma, bronchial hyper-reactivity, Korean population

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Introduction

Asthma is a common and heterogeneous respiratory disease characterized by intermittent airways obstruction and respiratory symptoms that are caused by chronic airway inflammation and remodelling [1]. This airway inflammation and remodelling is one of the main causes of bronchial hyper-responsiveness (BHR), which characterizes the pathophysiology of asthma. The remodelling process has been linked to aberrant epithelial–mesenchymal signalling and proliferation of biosynthetically active myofibroblasts that represent precursors of smooth muscle [2].

The ADAM (a disintegrin and metalloproteinase) gene family, of which currently there are 34 members, is a subgroup of the zinc-dependent metalloproteinase superfamily [3, 4]. While biologic roles have been elucidated in some family members including roles in sperm–egg fusion, myogenesis and growth factor shedding, others are yet to have their function elucidated. A number of ADAM proteins have been shown to be expressed on the cell surface and to have protease activity [5–8]. Other functions of ADAM proteins include mediating cell–cell interaction through the disintegrin domain and mediating cell–cell fusion events [9, 10].

The gene encoding a disintegrin and metalloprotease 33 (ADAM33) has recently been identified as a susceptibility gene for asthma using a positional cloning strategy [11]. In a genomewide scan of 460 Caucasian families enriched for doctor-diagnosed asthma, evidence for linkage was found on 20p13 with a maximum logarithm of odds (LOD) of 2.94 at the marker D20S482 (12.1 cM) which further increased to 3.93 when BHR was included in the definition of asthma. Physical mapping and direct cDNA selection identified 40 genes in the

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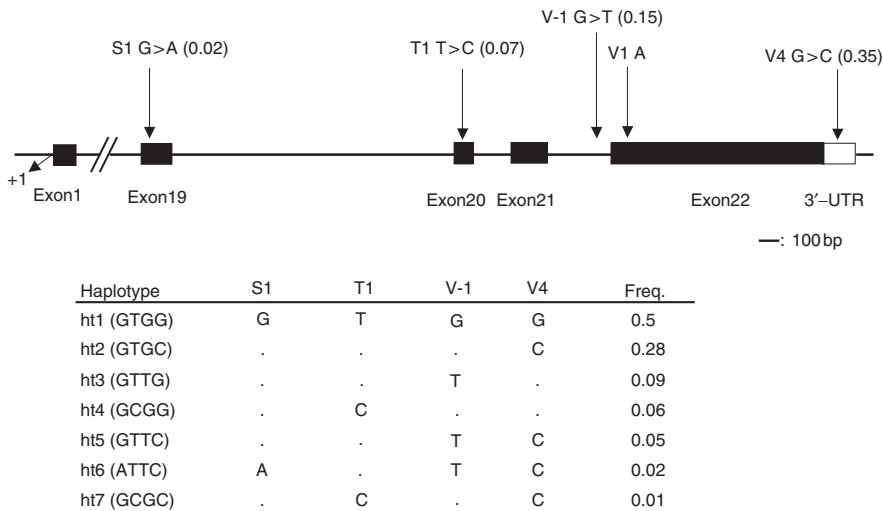


Fig. 1. Gene map and haplotypes of the ADAM33 gene. Coding exons are marked by shaded blocks and 3'-UTR by white block. Arrows indicate SNPs, which were genotyped in the whole study population ($n = 477$). First base of transcription start site was denoted as nucleotide +1. Dots in A2 represent the alleles that are found on the most common haplotype. Number in the parentheses represents the frequency of rare alleles.

region under the peak of linkage at 20p13. SSCP analysis and direct sequencing were used to identify single nucleotide polymorphisms (SNPs) in the region and 135 polymorphisms in 23 genes that lay in the 2.5 Mb (megabase) 90% confidence interval around the peak of linkage were typed (Fig. 1). Twenty-four SNPs were localized to a cluster of three genes showing significant association with both asthma and BHR. Fourteen of these lay within a single gene identified as ADAM33. The significance of ADAM33 as an asthma susceptibility gene is strengthened by the existence of a syntenic region on mouse chromosome 2 at 74 cM that has been linked to BHR [12] overlying an orthologue of ADAM 33 that exhibits 70% homology with its human counterpart [13].

The human and mouse genes for ADAM33 have now been identified and characterized. ADAM33 appears to be widely expressed in the placenta, developing embryo, skeletal muscle, small intestine, colon, heart and brain as well as the respiratory system. In the lung, ADAM33 is preferentially expressed in smooth muscle, myofibroblasts and fibroblasts, but not in epithelial cells, T cells or inflammatory leucocytes [13, 14]. From the initial study, it is unclear which SNP(s) in the ADAM33 gene are primarily responsible for the association observed with asthma and BHR due to the extensive linkage disequilibrium existing between the SNPs across the gene. It is also not apparent whether a gain or loss of function underlies the development of BHR and asthma. However, given the selective expression of ADAM33 in mesenchymal cells, it has been speculated that alterations in ADAM33 activity may underlie abnormalities in the function of airway smooth muscle cells and fibroblasts linked to airway remodelling and BHR [1].

While the initial study by Van Eerdewegh et al. has provided evidence for ADAM33 as a putative asthma susceptibility gene in outbred Caucasian populations, proof that this is truly an asthma susceptibility gene awaits replication in ethnically different populations. To assess whether genetic variants of ADAM33 gene are related to asthma in Korean population, we have conducted an association study of the known five polymorphisms of ADAM33 gene with asthma diagnosis and intermediate asthma phenotypes (BHR and serum total IgE) in a Korean asthmatic cohort.

Materials and methods

Subjects

Three hundred and twenty-six bronchial asthmatics and 151 normal controls were recruited from the Asthma Genome Research Center of Soonchunhyang University hospital. Ethical approval was obtained from the institutional review board of the hospital. All patients with asthma had current asthma symptoms, wheeze and dyspnea or cough, and met the American Thoracic Society definition of asthma [15]. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced (Salbutamol (VentolinTM MDI, GSK, Greenford, UK) 0.1 mg two times inhalation) improvement of more than 15% of forced expiratory volume in 1 s (FEV₁) and/or airway hyper-responsiveness as revealed by PC₂₀ (provocative concentration dose of methacholine producing a 20% fall in FEV₁) < 10 mg/mL of methacholine. A Tidal breathing method was utilized for the methacholine bronchial challenge. Forty-one subjects could not do methacholine bronchial challenge test, as they could not stop anti-asthmatic medications due to severe asthmatic symptoms. Normal subjects were recruited from spouse of the patients or the general populations. They were identified as those who answered negatively to a screening questionnaire for respiratory symptoms [16] and had FEV₁ ≥ 75% predicted, a PC₂₀ methacholine ≥ 10 mg/mL, a total serum IgE < 1000 IU/mL and a normal chest X-ray. Subjects were skin prick tested for 24 common inhalant allergens. Total IgE and specific IgE to *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp) were measured by CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as having a positive response (≥ 3 mm in diameter of weal reaction) to more than one allergens on skin prick test, or a positive response (score > 1) of specific IgE to Dp or Df. The clinical parameters are summarized in Table 1.

Genotyping by single base extension and electrophoresis

Five SNPs that were previously shown to be associated with asthma and BHR [11] were genotyped (216S1 G>A, 216T1 T>C, 216V-1 C>A, 216V1 T>A, 216V4 C>G) (Fig. 1) [11]. Primer extension reactions were performed with SNaP-

Table 1. Clinical characteristics

	Asthma	Control
Number of subjects	326	151
Age: median (range)	48 (11–78)	27 (10–74)
Sex (male/female)	131/195	80/71
Smoker (%)	32.6	37.4
Smoking (pack/year)	21.9 ± 17.9	11.0 ± 7.8
FVC (% predicted)	85.6 ± 18.8	88.7 ± 11.4
FEV ₁ (% predicted)	76.7 ± 21.9	94.4 ± 12.2*
% changes of FEV ₁ by bronchodilator	10.8 ± 10.6	3.2 ± 4.9*
PC ₂₀ (mg/mL)	3.3 ± 5.9	23.5 ± 3.8*
Total IgE (IU/mL)	352 ± 626	129 ± 165*
Positive of skin test: N (%)	108/326 (33.1)	30/151 (19.9)*
Positive of specific IgE: Dp	118/326 (36.2)	47/151 (31.1)
Positive of specific IgE: Df	145/326 (44.5)	54/151 (35.8)
Atopy: N (%)	173/326(53.1)	64/151 (42.4)**

Mean ± standard deviation. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; Dp, *Dermatophagoides pteronyssinus*; Df, *D. farinae*. **P*-value < 0.01. ***P*-value < 0.05, compared between asthma and control.

shot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA) essentially according to the manufacturer's recommendation. Primer extension reactions were cleaned through addition of 1 U, Shrimp Alkaline Phosphatase at 37 °C for 1 h, followed by 15 min at 72 °C. DNA samples, containing extension products, and Genescan 120 Liz size standard solution was added to Hi-Di formamide (Applied Biosystems) as recommend by the manufacturer, incubated at 95 °C for 5 min, followed by 5 min on ice and then electrophoresed on an ABI Prism 3100 Genetic Analyzer. Data were analysed using ABI Prism GeneScan 3.5.1 and Genotyper 3.6 software (Applied Biosystems).

Statistics

The distribution of allele frequencies were tested for confirmation to Hardy-Weinberg equilibrium by χ^2 tests. Linkage disequilibrium between loci were measured using Lewontin's $|D'|$ [17]. Haplotypes and their frequencies were inferred using the algorithm developed by Excoffier et al. [18] implemented in the software Arlequin (Genetics and Biometry Laboratory, Geneva, Switzerland). The allelic and haplotypic frequencies in the asthmatics and normal control populations were compared using logistic regression analysis. Differences of logarithms of PC₂₀ levels and IgE level among genotypes or haplotypes were examined using a general linear model in asthmatics. Before multiple analyses, simple linear regression was carried out between log-transformed PC₂₀ levels and possible co-variants such as age, sex, presence of atopy and smoking history. All statistical analysis was performed using software SAS (version 8.1, Cary, NC, USA).

Results

A map of the ADAM33 gene and predicted haplotypes is presented in Fig. 1. Of the five loci genotyped, the V1 SNP was non-polymorphic with the frequency of the A allele being 1.0 in the Korean population compared to the previously reported frequency of 0.98 in a Caucasian non-asthmatic

Table 2. Linkage disequilibrium of locus by locus

	S1	T1	V-1	V4
S1	–	1	1*	1*
T1	0.02	–	1*	0.82*
V-1	0.11	–0.1	–	0.19
V4	0.05	–0.1	–0.05	–

Up-right areas are Lewontin's $|D'|$ and down-left areas are d from Kaplan and Weir.

**P*-value < 0.001 of D' from 0, by χ^2 test.

population [11]. As a consequence, V1 was excluded from the association analysis. The distributions of SNPs of all four loci were in Hardy-Weinberg equilibrium (HWE; $P > 0.05$, data not shown) in the normal controls. The allele frequency of the V4 locus SNP ($G = 0.65$) in the present study population was significantly different to that previously reported in a Caucasian population ($G = 0.23$) [11]. Therefore, in the present study, data were analysed using V4 C as the rare allele (Fig. 1).

Linkage disequilibrium between SNPs, locus by locus was examined and the results are presented in Table 2. Seven haplotypes were constructed by the EM algorithm implemented in the Arlequin program and their frequencies are listed in Fig. 1. One haplotype (ht6) was a pseudohaplotype, differing from ht5 by only the S1 SNP, and thus was excluded from the analysis. The frequencies of the SNPs and haplotypes are shown in Table 3. Allele frequencies of each SNP and haplotypes were compared between the patients and the normal controls using logistic regression models with the covariates of age and sex (Table 3). There was no significant difference in the distribution of SNPs and the six haplotypes between asthmatics and normal controls. All single SNPs and six haplotypes in ADAM33 were also analysed for association with PC₂₀ using general linear models with Type III SS in the asthmatic patients (Table 4). The distribution of the T1 alleles and one haplotype (ht4: GCGG) showed a significant association with log-transformed PC₂₀ methacholine in the asthma patients ($P = 0.03$ and 0.0007, respectively, using a co-dominant model, Table 4). There was no association seen between any SNP or haplotype and PC₂₀ in the normal controls. However, the sample size for this analysis was much smaller than that of the asthmatic cohort ($N = 151$ vs. $N = 326$) and the degree of BHR much lower and thus may represent type II error. There was no SNP or haplotype associated with log-transformed total IgE levels (data not shown).

Discussion

The members of the ADAM gene family encode type-1 transmembrane proteins with a unique domain structure composed of a signal sequence and the following domains: pro, metalloprotease, disintegrin, cystein-rich, epidermal growth factor (EGF)-like, transmembrane and cytoplasmic [7, 19]. Over 30 ADAM family members have been identified, but their biological roles have been elucidated in only a few. Tumor necrosis factor- α (TNF- α) converting enzyme (TACE/ADAM17) was found to be a protease required for shedding active TNF- α from the cell surface [20, 21] by cleaving pro-TNF- α . More recently, it has been found that ADAM 17 is

Table 3. Allelic and haplotypic distributions of *ADAM33* gene between patients with asthma and normal controls in Korea

		N (frequency)			Frequency	P-value	Odds (95% CI)
Polymorphism		C/C	C/R	R/R	Rare allele		
S1	BA	299 (0.96)	12 (0.04)	0 (0)	0.02	0.78	1.16 (0.4–3.37)
	NC	145 (0.97)	5 (0.03)	0 (0)	0.02		
T1	BA	265 (0.84)	48 (0.15)	2 (0.01)	0.07	0.94	1.02 (0.62–1.69)
	NC	125 (0.84)	22 (0.15)	1 (0.01)	0.08		
V-1	BA	228 (0.75)	60 (0.2)	15 (0.05)	0.16	0.21	1.29 (0.87–1.9)
	NC	115 (0.8)	25 (0.17)	4 (0.03)	0.11		
V4	BA	125 (0.4)	146 (0.47)	39 (0.13)	0.36	0.59	0.92 (0.69–1.24)
	NC	61 (0.42)	71 (0.48)	15 (0.1)	0.34		
		Ht ⁻ /Ht ⁻	Ht ⁺ /Ht ⁻	Ht ⁺ /Ht ⁺	Haplotype		
Ht1	BA	51 (0.18)	164 (0.57)	74 (0.26)	0.47	0.25	0.83 (0.61–1.14)
	NC	19 (0.14)	81 (0.57)	41 (0.29)	0.54		
Ht2	BA	173 (0.6)	90 (0.31)	26 (0.09)	0.21	0.98	1.01 (0.74–1.37)
	NC	83 (0.59)	47 (0.33)	11 (0.08)	0.23		
Ht3	BA	261 (0.9)	25 (0.09)	3 (0.01)	0.05	0.66	1.15 (0.62–2.15)
	NC	129 (0.92)	11 (0.08)	1 (0.01)	0.04		
Ht4	BA	264 (0.91)	24 (0.08)	1 (0)	0.04	0.52	1.28 (0.61–2.68)
	NC	131 (0.93)	10 (0.07)	0 (0)	0.03		
Ht5	BA	252 (0.87)	37 (0.13)	0 (0)	0.06	0.19	1.58 (0.8–3.13)
	NC	129 (0.92)	12 (0.09)	0 (0)	0.04		
Ht7	BA	270 (0.93)	19 (0.07)	0 (0)	0.03	0.64	0.83 (0.38–1.8)
	NC	130 (0.92)	11 (0.08)	0 (0)	0.04		

BA, bronchial asthma, NC, normal control, C/C, C/R and R/R represent homozygotes for common alleles (C/C), heterozygotes (C/R) and homozygotes for rare alleles (R/R). The presence of haplotype designates Ht⁺. Rare allele and haplotype is dominant and recessive in dominant and recessive models, respectively. *P*-values for logistic analysis of co-dominant models controlling age and sex as co-variables. Haplotypes and their frequencies were inferred using algorithm developed by Excoffier et al. (18). Ht6 was not analysed because it was equivalent model with S1 (Fig. 1).

Table 4. Regression analysis log(PC₂₀) with four single nucleotide polymorphisms and haplotypes among asthma patients

		N (Mean ± SD)			P-value		
Polymorphism		C/C	C/R	R/R	Co-dominant	Dominant	Recessive
S1		260 (0 ± 0.04)	10 (0.25 ± 0.2)	0 (±)	0.25	0.59	
T1		234 (0.02 ± 0.04)	39 (–0.17 ± 0.11)	1 (1.4 ±)	0.03	0.43	0.22
V-1		198 (0.02 ± 0.05)	50 (0.02 ± 0.08)	14 (–0.38 ± 0.19)	0.1	0.23	0.06
V4		108 (–0.03 ± 0.07)	124 (0.02 ± 0.06)	37 (0.06 ± 0.08)	0.75	0.88	0.88
		Ht ⁻ /Ht ⁻	Ht ⁺ /Ht ⁻	Ht ⁺ /Ht ⁺			
Ht1		47 (–0.01 ± 0.09)	140 (0.02 ± 0.06)	63 (0.03 ± 0.09)	0.59	0.79	0.84
Ht2		146 (–0.04 ± 0.06)	79 (0.11 ± 0.08)	25 (0.01 ± 0.11)	0.32	0.95	0.85
Ht3		225 (0.04 ± 0.04)	23 (–0.17 ± 0.15)	2 (–0.47 ± 0.36)	0.43	0.78	0.59
Ht4		229 (0.02 ± 0.04)	20 (–0.14 ± 0.16)	1 (1.4 ±)	0.0007	0.6	0.24
Ht5		217 (0.03 ± 0.05)	33 (–0.05 ± 0.1)	0 (±)	0.24	0.32	0.87
Ht7		236 (0.03 ± 0.04)	14 (–0.2 ± 0.15)	0 (±)	0.17	0.13	

Genotype and haplotype distributions, means, and standard deviations (SD) of log (PC₂₀ methacholine), and *P*-value of 3 alternative models for regression analysis are shown. The presence of haplotype designates Ht⁺. Rare allele and haplotype is dominant and recessive in dominant and recessive models, respectively.

required for the shedding of other cell surface proteins including L-selectin and TNF- α [22]. The role of ADAM proteins and effects of ADAM genes polymorphisms in the pathobiology of asthma have not been revealed thoroughly. A loss of function mutation could impair the shedding of growth factor receptors, where a gain of function mutation could enhance the shedding of growth factors, which lead to

increased inflammation or a shift towards an immune response mediated by type 2 helper T cells [23].

In UK and US Caucasian populations, the S1, T1, V-1, V1 and V4 polymorphisms were significantly associated with asthma ($P = 0.04 - 0.003$). In our population, V1 was non-polymorphic compared to the previously observed frequency of the rare allele of 1.9% in the asthmatics and 3.5% in the

controls of the previously reported Caucasian population [11]. As multiple SNPs may act in combination to increase the risk of asthma, haplotypes were constructed and their frequencies compared between asthmatics and controls. Analysis of the association of the other four SNPs and six haplotypes with asthma phenotype did not provide evidence that any SNP or haplotype of ADAM33 gene tested were associated with asthma development. However the distribution of both the T1 SNP ($P=0.03$) was associated with higher BHR in asthmatics and ht4 (GCGG) was strongly associated with the levels of PC₂₀ in response to methacholine in the asthmatic population ($P=0.0007$). The association of the T1 SNP may reflect the fact that it is a proxy marker for ht4 or possibly could be artefactual as only one subject was homozygote for the rare allele of T1.

The rare allele of T1 (T1 C) was increased in frequency in asthmatics compared to normal controls of the previously studied Caucasian population (24.1% vs. 7.4%) [11], while the frequency was similar in the both groups of our study (9% vs. 8%). One explanation for the discrepancy between the studies may be the difference in study populations. Caucasian controls were collected in both the US and the UK and 130 IBD-affected individuals (identical by descent for chromosome 20p13) and 217 'hyper-normal' (negative both for a personal and familial history of asthma) controls were compared for allele and genotype frequencies. In our study, 326 cases were recruited from referred patients using strict criteria for asthma, such as bronchodilator test ($>15\%$ of FEV₁) and/or PC₂₀ methacholine. Normal controls in the present study included atopic controls (42.2% in Table 1), who showed positive skin tests and specific IgE antibody to one or two house dust mites. The prevalence of non-atopic asthma was higher than typically seen in western populations. These differences in subject characteristics may account for the lack of observed association of the ADAM33 gene with asthma susceptibility in the present study. Alternatively, the lack of replication may be accounted for ethnic differences between the study populations as suggested by recent investigations [24–26].

The substitution of thymine for cytosine in the T1 SNP results in the substitution of methionine with threonine in the cytoplasmic domain of the protein. This could potentially alter intracellular signalling, resulting in increased fibroblast and smooth muscle proliferation. Further study is required to elucidate precisely which SNPs in ADAM33 alter the genes expression and/or the function of its product. Such studies should enhance our understanding of the disease process and lead to new therapeutic targets for asthma.

In conclusion, we suggest a possible association of ADAM33 with BHR (but not asthma *per se*) in a non-Caucasian population sample. This adds to the increasing body of evidence suggesting that ADAM33 plays an important role in the pathogenesis of airways hyper-responsiveness, one of the cardinal features of asthma.

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