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ADAM33 polymorphisms are associated with asthma susceptibility in a Japanese population

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Summary

Background Asthma is the most common chronic disorder in childhood, and asthma exacerbation is an important cause of childhood morbidity and hospitalization. Asthma is believed to be a complex disorder involving genetic and environmental factors, and several asthma susceptibility loci have been identified through genome-wide screening. A disintegrin and metalloprotease 33 (ADAM33) was the first asthma susceptibility gene to be discovered by positional cloning in 2002.

Objective The aim of the present study was to investigate whether single-nucleotide polymorphisms (SNPs) in ADAM33 are associated with childhood asthma in the Japanese population.

Methods Twenty-three ADAM33 SNPs were genotyped by fluorescence correlation spectroscopy with the use of DNA from 155 families (538 members) identified through children with atopic asthma. The transmission disequilibrium test (TDT) was performed for family-based association study.

Results TDT revealed that minor alleles of S+1, ST+4, and T2 SNPs were over-transmitted to asthma-affected offspring ($P < 0.05$). According to the haplotype TDT, no haplotype of ADAM33 was transmitted preferentially to asthmatic offspring.

Conclusion Our results confirm the involvement of ADAM33 in the development of childhood asthma among the Japanese.

Keywords ADAM33, childhood asthma, SNP, TDT

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Introduction

Asthma is the most common chronic disorder in childhood, and asthma exacerbation is an important cause of childhood morbidity and hospitalization. Overall, asthma affects nearly 155 million individuals worldwide [1].

Atopy, which is characterized by increased levels of IgE against common environmental allergens, is considered the strongest predisposing factor for asthma. Family and twin studies have shown that atopy runs in families [2–4]. More than 90% of children with asthma develop specific IgE against the house dust mites, and dust-mite allergy is strongly associated with asthma [5, 6]. However, only a small subset of subjects with dust-mite allergy develops asthma [7], suggesting that other factors are necessary for the development of asthma. Another characteristic feature

of asthma is bronchial hyper-responsiveness (BHR). BHR involves altered airway structure and wall thickening, and thickened asthmatic airways account for a large proportion of cases of BHR and excessive airway narrowing. Previous studies have shown that BHR is inherited independently of atopy [8, 9], suggesting that genes involved in BHR differ from those involved in atopy. These data suggest that genes involved in BHR in addition to genes for atopy play important roles in the development of asthma.

Asthma is believed to be a complex disorder involving genetic and environmental factors, and several asthma susceptibility loci have been identified through genome-wide screening [10–16]. A gene encoding a disintegrin and metalloprotease 33 (ADAM33) was the first asthma

susceptibility gene to be discovered by positional cloning in 2002 [17]. Since 2002, a number of replication studies have been conducted in diverse ethnic populations and in patients with different clinical manifestations. Some single-nucleotide polymorphisms (SNPs) in *ADAM33* have been associated with asthma, but others showed conflicting results (reviewed in reference [9]). Recently, Blakey et al. [18] performed a meta-analysis for *ADAM33* and asthma and reported that the F+1 and ST+7 variants in *ADAM33* were significantly associated with asthma in case-control and family-based studies. The role of *ADAM33* in the development of asthma remains unclear, but the accumulating data indicate that *ADAM33* may be involved in airway remodelling and may determine lung function throughout life [9]. To date, most data available for *ADAM33* and asthma have been obtained from Caucasians, and few data on *ADAM33* SNPs associated with asthma and atopic disease are available for Asian populations [19, 20].

The aim of the present study was to determine whether *ADAM33* SNPs are associated with the development of asthma in the Japanese. We genotyped previously reported *ADAM33* polymorphisms and examined possible associations of these polymorphisms with atopic asthma in Japanese families identified through asthmatic children.

Materials and methods

The probands were mite-sensitive asthmatic children who visited the Paediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the study was given to all family members interviewed, and 155 families (538 members), including 47 families used for our genome-wide screening [16], gave informed consent and participated in this study.

Each family member was questioned regarding allergic symptoms and underwent a physical examination performed by a participating paediatrician. Asthma was diagnosed in subjects according to the criteria of the National Institutes of Health, USA [21]. Patients had to satisfy the following criteria: (1) two or more episodes of wheezing and shortness of breath during the past year and (2) reversibility of the wheezing and dyspnoea, either spontaneously or by bronchodilator treatment. Methacholine challenge testing was not performed because of the low ages of the asthmatic patients in the families; however, asthma was diagnosed differentially in the affected children by participating physicians or paediatricians who had treated the children for more than 2 years. Because wheezing is often associated with viral respiratory infection in young children, we excluded children younger than 3 years of age [22]. The young adult patients included in this study had chronic asthma since childhood. Total serum IgE levels and IgE levels specific for *Dermatophagoides farinae* (Df) were determined with the

Table 1. Clinical details of the asthmatic families

No. of families	155
No. of affected children	228
Mean age (years $\pm$ SD)	10.1 $\pm$ 5.8
Total IgE (IU/mL $\pm$ SD)	1254, 35–19 000
Df-RAST IgE (UA/mL $\pm$ SD)	63, 3.9–100
No. of parents	310
Mean age (years $\pm$ SD)	40.2 $\pm$ 7.1
Total IgE (mean IU/mL, range)	287, 0–5200
Df-RAST IgE (mean UA/mL, range)	8.5, 0–100

Values < 0.34 and > 100 for Df RAST IgE were expressed as 0 and 100, respectively, in this study.

Pharmacia CAP System (Uppsala, Sweden). To achieve homogeneity of the asthmatic patients, we selected families having more than one asthmatic child with a Df RAST score > 3 . The clinical characteristics of the families are shown in Table 1.

DNA was extracted from peripheral blood leucocytes. Genotyping of 23 *ADAM33* SNPs was performed by fluorescence correlation spectroscopy (FCS) [23]. In brief, first-round PCR was performed to amplify a fragment containing an SNP site. The first-round PCR products were used for sequence-specific primer-polymerase chain reaction (SSP-PCR). In SSP-PCR, allele-specific primers, which differ in a single nucleotide at the 3' end and are coupled with different fluorescence dyes (TAMRA and Cy5), were used for allele-specific amplifications. SSP-PCR with two allele-specific primers was performed competitively in a 10 μ L reaction mixture consisting of 0.5 U AmpliTaq DNA Stoffel Fragment (Applied Biosystems, Foster City, CA, USA), 200 μ M dNTP, 2.5 mM MgCl₂, 20 nM each primer, with 0.5 μ L of first-round PCR products. Amplified products were subjected to FCS at 543 and 633 nm excitation wavelength with a single molecule fluorescence detection system (MF10, Olympus, Tokyo, Japan). Primers used for first-round PCR and SSP-PCR are listed in Table 2. Accuracy of genotyping was confirmed by the sequence of at least 16 unrelated individuals.

Statistics

Deviation from Hardy–Weinberg expectancy was examined with the χ^2 -test. Differences in allele frequencies were analysed with the χ^2 -test. The association between total serum IgE levels and genotypes was analysed by ANOVA and Wilcoxon's test. The transmission disequilibrium test (TDT) was performed with the ASPEX Program [24]. Quantitative TDT analysis for total serum IgE was performed with QTDT software [25]. Linkage disequilibrium (LD) between SNPs, as expressed by D prime, was calculated with Haploview software [26]. Haplotypes were inferred by using an accelerated EM algorithm [27], which was implemented in Haploview [26]. Haplotype association testing was performed with Haploview software [26]. Haplotype blocks were defined by the method of Gabriel et al. [28]. Statistical

Table 2. Primers used for single-nucleotide polymorphism (SNP) genotyping

SNPs	db SNP ID	First primer (F)	First primer (R)	Primer A (TAMRA)	Primer B (Cy5)
F1	rs3918392	ATACTGGGACTCGAGGCTG	ACCTAGTGGCTGATCACC	ATC CCT GTG GCC ACA GGT	ATC CCT GTG GCC ACA GGC
F+1	rs511898	TGTACAGTTCAGGTACTTCCG	AGATCTTTTCGGATGGAGCAG	CGA GGC CTG TGA ATT CCC	CGA GGC CTG TGA ATT CCT
G-1	rs2485700	TGTACAGTTCAGGTACTTCCG	AGATCTTTTCGGATGGAGCAG	TGA GCC CTG ACC ACC AAC	TGA GCC CTG ACC ACC AAT
L-1	rs2280092	GGCGAGCCGACTTAACCTGG	TTTCCGAGTAACTGGCGAGG	AGA GAA GCG CGG GGG TTG GG	AGA GAA GCG CGG GGG TTG GA
M+1	rs3918395	GGAGCCAGGCTACCCAAGC	CCCTGGCCAGGTTAAGTCGG	GCC GGC TCC CAA GCT CCC	GCC GGC TCC CAA GCT CCA
Q-1	rs612709	GAAGCTCTGGAAGGCATTC	TAGATGGCCAGGAAGTGACTTG	TTC AAA CGG CAA GGA GGA	TTC AAA CGG CAA GGA GGG
S1	rs3918396	AGGAGTAGGCTCAGGAAGCAGG	TGCAGGCTGAAAGTATGCCAG	GAG CAG AGG CAG CAG GAT	GAG CAG AGG CAG CAG GAC
S2	rs528557	CAGGAGTAGGCTCAGGAAGCAG	TGCACCTGCTCAGGACTCAG	CTG CCT CTG CTC CCA GGG	TGC TAA CTC TGC TCC CAG GC
S+1	rs2853209	GAAGTCAAGGGTGAGGCAGC	TGCTTCTGAGCTACTCTTG	GGC CTC CCA GTC AAG CGT	GGC CTC CCA GTC AAG CGA
ST+4	rs44707	TCTAGGACAGCCATGCTTAG	TCAGATTGCAGTCCCTGTACC	CAG CAT TTG GGA ACT TCA AGT	AGC ATT TGG GAA CTT CAA GG
ST+7	rs574174	CAAAGAACCTTGGATGTCGG	TGCTGACATAGCTGGTCTTG	ACT GTC CCC ATC CCA TCC	ACT GTC CCC ATC CCA TCT
T1	rs2280091	GACTCAAGGTGACTGGGTGC	TAAACCACTCAGGATCACTGTG	GGG CGG CGT TCA CCC CAT	GGG CGG CGT TCA CCC CAC
T2	rs2280090	GACTCAAGGTGACTGGGTGC	TAAACCACTCAGGATCACTGTG	CTC ACC CAG GGG CCA GGA	CTC ACC CAG GGG CCA GGG
T+1	rs2280089	TGAATCCAGGTCCTGCTCTAC	TCTTATCACGTTGCTCAGCCC	GCT CAT GCC TCC TGC CTT	GCT CAT GCC TCC TGC CTC
T+2	rs630712	TGAATCCAGGTCCTGCTCTAC	TCTTATCACGTTGCTCAGCCC	GTG AAT ATG GTC AGC AGG AGA	TGA ATA TGG TCA GCA GGA GC
V-3	rs628977	TTCTGGGACTGATGGTTTAT	GGCTCAGGTTATTATGGAAA	GTG TGG CAG IGA GCC CAC	GTG TGG CAG IGA GCC CAT
V-2	rs628965	TTCTGGGACTGATGGTTTAT	GGCTCAGGTTATTATGGAAA	TGC AAG CTG TGT GGC AGA	TGC AAG CTG TGT GGC AGG
V-1	rs543749	TTCTGGGACTGATGGTTTAT	GGCTCAGGTTATTATGGAAA	GGA GGA TAT GTT GTC CCC TAA G	AGG AGG ATA TGT TGT CCC CTA AT
V1	rs3918401	TTCTGGGACTGATGGTTTAT	GGCTCAGGTTATTATGGAAA	TCC TCA TTC TCA GCA GAT CAA	CTC CTC ATT CTC AGC AGA TCA T
V2	rs3918400	CCGTGGAAATTCATGTAGG	TTAAAGACAGGTGGCCACTGAC	CCT GGA GTG GGT GGG TCG	CCT GGA GTG GGT GGG TCA
V3	rs677044	CCGTGGAAATTCATGTAGG	TTAAAGACAGGTGGCCACTGAC	AGG AAC CCA GAG CCA CAT	AGG AAC CCA GAG CCA CAC
V4	rs2787094	GAGCTCCAGAACTCAGGTG	CTACATGCAATTTCCAGGAC	GAG TCC ACA CTC CCC TGG	GAG TCC ACA CTC CCC TGC
V5	rs3746631	GAGCTCCAGAACTCAGGTG	CTACATGCAATTTCCAGGAC	CTG GCT GGC CTC TGC AAG	CTG GCT GGC CTC TGC AAA

Table 3. Results of transmission disequilibrium test (TDT) in single nucleotide polymorphisms (SNPs)

SNPs	db SNP ID	SNPs	Allele*	Allele frequencies				Transmitted	Not Transmitted	P-Values
				Present study	Van Eerdewegh et al. [17]	Cheng et al. [19]	Lee et al. [20]			
F1	rs3918392	A/G	G	0	0.032 [†]			41	44	0.8
F+1	rs511898	C/T	T	0	0.35	0.38		95	102	0.64
G-1	rs2485700	C/T	C	0	0.093 [†]			50	44	0.58
L-1	rs2280092	C/T	T	0	0.11	0.14		40	32	0.4
M+1	rs3918395	G/T	T	0	0.11			51	37	0.14
Q-1	rs612709	A/G	A	0	0.15			40	49	0.25
S1	rs3918396	A/G	A	0	0.105 [†]		0.02 [†]	2	1	1
S2	rs528557	C/G	G	0	0.26	0.3		75	82	0.64
S+1	rs2853209	A/T	T	0	0.49			135	98	0.012
ST+4	rs44707	A/C	C	0	0.49			117	90	0.038
ST+7	rs574174	C/T	T	0	0.12			45	42	0.81
T1	rs2280091	A/G	G	0	0.11	0.15	0.08 [†]	35	30	0.42
T2	rs2280090	A/G	A	0	0.094 [†]	0.18		62	42	0.03
T+1	rs2280089	A/G	A	0	0.11	0.15		48	36	0.2
T+2	rs630712	A/C	C	0.27	0.117 [†]			91	74	0.21
V-3	rs628977	C/T	T	0	0.37			99	93	0.69
V-2	rs628965	A/G	A	0	0.37			101	87	0.26
V-1	rs543749	G/T	T	0	0.15		0.11	57	52	0.56
V1	rs3918401	A/T	T	0	0.035 [†]			43	50	0.52
V2	rs3918400	A/G	A	0	0.037 [†]			56	56	1
V3	rs677044	A/G	G	0	0.22			80	80	1
V4	rs2787094	C/G	G	0	0.233 [†]		0.34	97	100	0.89
V5	rs3746631	C/T	C	0	0.037 [†]			49	55	0.57

*Alleles are based on reference SNP number.

[†]Allele frequencies that are significantly different from those in the present study ($P < 0.05$).

significance was defined at the 5% level. The statistical power of the TDT for detecting a polymorphism that directly affected risk was calculated according to the method proposed by Risch and Merikangas [29].

Results

We genotyped 23 SNPs in *ADAM33*. The allele frequencies of the parental genotypes for all SNPs did not deviate from expected values based on Hardy–Weinberg equilibrium ($P > 0.05$). Minor allele frequencies of SNPs are shown in Table 3. Allele frequencies of nine SNPs in the present study differed significantly from those reported in Caucasians [17] ($P < 0.05$, Table 3). The allele frequencies of controls in another Japanese population [19] (Table 3) did not differ from those in the present study ($P > 0.1$), but the S1 and T1 allele frequencies in Koreans (Lee et al. [20], Table 3) differed significantly from those in the present study ($P = 0.005$ for S1 and $P = 0.005$ for T1). Although Koreans are considered genetically close to Japanese, our data indicate that there are considerable ethnic differences in *ADAM33* allele frequencies. The LD map constructed from 22 SNPs with minor allele frequencies > 0.05 is shown in the Fig. 1, as measured by D prime. The allele frequency of S1 was very low (0.003) in

our population, so this SNP was dropped from the analysis. Tight LD was observed throughout the region. Haplotypes with frequencies > 0.01 are shown in Table 4.

The TDT revealed that minor alleles of S+1, ST+4 and T2 SNPs were over-transmitted to asthma-affected offspring ($P < 0.05$, Table 3). The statistical power of the TDT for *ADAM33* SNPs without S1 was more than 0.8 at the α level of 0.05 if the relative risk for asthma in those persons carrying a putative risk allele is 1.8 compared with that in persons without the allele. No association was observed between total serum IgE levels and any *ADAM33* SNP according to quantitative TDT analysis ($P > 0.05$). Mean log (total serum IgE) levels of parents with each genotype in a single SNP were compared, and no significant association was observed between groups ($P > 0.05$).

According to the haplotype TDT, no haplotype of *ADAM33* was transmitted preferentially to asthmatic offspring (Table 4). As shown in Fig. 1, ST+4, which was associated with the development of asthma ($P = 0.038$), was not included in any of the haplotype blocks.

Discussion

In the present study, we observed that minor alleles of *ADAM33* SNPs were significantly over-transmitted to

atopic-asthma-affected offspring, confirming the previous studies showing that *ADAM33* is associated with asthma. The allele frequencies in the present study were similar to those reported by Cheng et al. [19] but differed significantly from those reported in Caucasians and Koreans. A lesser degree of difference in allele frequencies was detected between our subjects and reported Korean subjects than between our subjects and reported Caucasian subjects.

Correction for multiple tests, such as Bonferroni's correction, is used to exclude a high probability of false-positive results. However, the aim of the present study was

to confirm associations between *ADAM33* polymorphisms and asthma in our population, and correction such as Bonferroni's correction seems to be too stringent. Moreover, SNPs were in tight LD in this population, and they are not independent of each other. We therefore applied *P*-values of 0.05 in the present study.

ADAM33 SNPs have been shown to be associated with an accelerated decline in lung function of asthmatics followed up for over 20 years [30]. Moreover, Simpson et al. [31] reported that *ADAM33* SNPs were associated with reduced lung function at the age of 3 years (F+1) and with decreased FEV1 (F+1, M+1, T1, and T2), suggesting that reduced early-life lung function is in part genetically determined. In the present study, all cases were of childhood or child-onset asthma, supporting the idea that *ADAM33* SNPs may be associated with smaller airways.

The study by Van Eerdewegh was the first to show that *ADAM33* polymorphisms (Q – 1, S1, ST+4, ST+7, V – 1, and V4) and haplotypes were associated with asthma in Caucasian families [17]. A number of studies have been conducted to replicate the original findings, some confirming and others refuting the association of *ADAM33* with asthma [32–35]. A meta-analysis of *ADAM33* SNPs and asthma showed that F+1 and ST+7 were significantly associated with asthma in both TDT and case–control studies [18]. Most of the data in the meta-analysis were from Caucasian subjects; only one study of Asian origin [20] was included. In the present study, childhood asthma was associated with *ADAM33* SNPs, S+1, ST+4, and T2, but not with F+1 and ST+7. The

Table 4. Haplotypic transmission test

Haplotype		Frequencies	Transmitted/ not transmitted	<i>P</i> -value
Block 1				
V1, V2	AG	0.84	46.3 : 35.0	0.21
	TA	0.12	27.0 : 33.0	0.44
	AA	0.042	11.0 : 15.3	0.40
Block 2				
T+1, T+2, V – 3,	GACGG	0.38	66.1 : 79.5	0.27
V – 2, V – 1	GCTAG	0.26	59.1 : 53.0	0.57
	GACGT	0.15	37.0 : 36.0	0.91
	AACGG	0.12	32.6 : 24.2	0.26
	GATAG	0.077	19.0 : 26.2	0.28
Block 3				
ST+7, T1, T2	CAG	0.72	59.4 : 74.4	0.19
	CGA	0.14	41.1 : 29.9	0.18
	TAG	0.12	31.0 : 31.6	0.94
	CAA	0.021	8.9 : 4.3	0.21

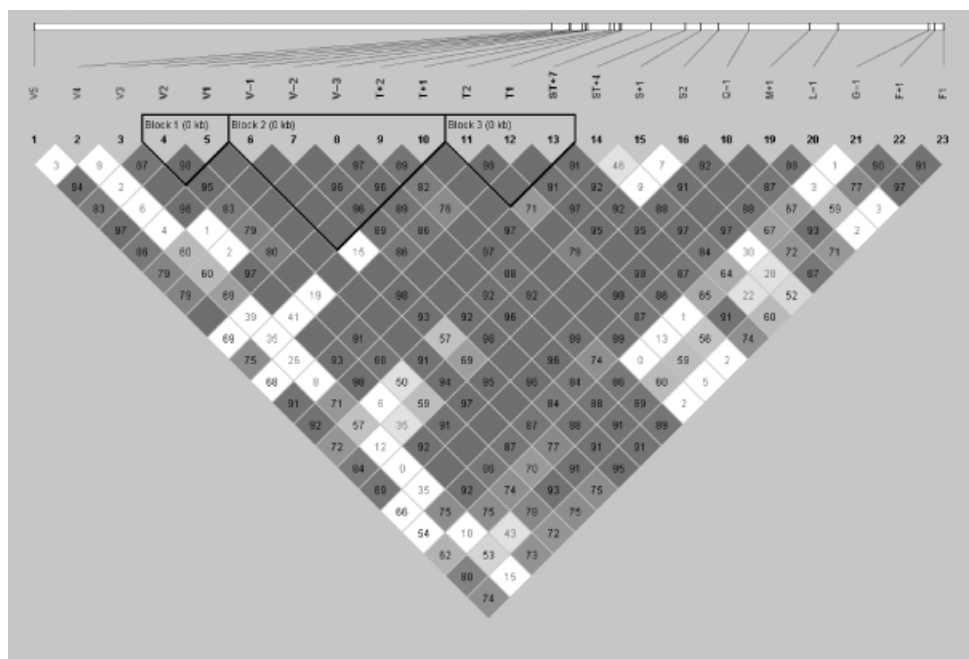


Fig. 1. Pairwise linkage disequilibrium (LD) between SNPs in *ADAM33* as measured by D prime in 538 subjects (155 families). LD is divided into three blocks. The D prime values for LD are colour-coded and the extent of red indicates the strength of LD.

genetic basis of asthma may differ between different ethnic groups; it is possible that a particular subset of SNPs may be a risk factor for asthma in Caucasians, and that a different subset may increase the risk in Asian populations.

Recently, Raby et al. performed a family-based association study for childhood asthma and ADAM33 SNPs in 652 nuclear families. They showed that no single SNP was associated with childhood asthma in Caucasians but that a common haplotype of ADAM33 was associated with the disease [35]. They also reported that T1 and T+1 were marginally associated with childhood asthma in Hispanics [35]. In previous association studies of ADAM33 and asthma/asthma-associated phenotypes, no single ADAM33 SNP was consistently associated with asthma or intermediate phenotypes. This may be because of allelic heterogeneity, a single disorder caused by different mutations within a gene. Another explanation is the difference in LD patterns between populations, which means that unobserved causal variants are the same in different populations, but that SNPs in strong LD with the variants differ between populations or that the disease-causing variants are in strong LD with an SNP in one ethnic population, but not in others. The allele frequencies in the present study were similar to those reported in another Japanese study [19] but differed significantly from those reported in Caucasians [17] and Koreans [20]. Suggesting that different genetic backgrounds may account for the discrepant results.

In recent years, much has been performed to catalogue millions of SNPs and haplotypes across diverse populations and to use these to identify subsets of highly informative tag SNPs for genetic association studies (HapMap projects, <http://www.hapmap.org/>). Haplotypes are considered to carry information about possibly unobserved causal variants in the region. In the present study, SNPs, S+1, ST+4, and T2 were associated with asthma but not with any haplotype, implying that S+1, ST+4, and T2 themselves or variants in strong LD with these are disease-causing variants in this population.

ADAM is a transmembrane protein that contains a disintegrin and metalloprotease domain, and, therefore, it potentially has both cell adhesion and protease activities. ADAM33 is a member of the ADAM family, which includes 35 proteins, and it belongs to the ADAM12, 15, and 19 subfamilies, which exhibit proteolytic activity [36]. Human ADAM33 is expressed in all tissues except liver [37], and an *in situ* hybridization experiment showed preferential expression of ADAM33 in smooth muscle, myofibroblasts and fibroblasts of asthmatic airways, but not in epithelium, endothelium, T cells or inflammatory cells [9]. Although the exact roles of ADAM33 in the development of asthma and BHR remain unclear, accumulated association data support the conclusion that ADAM33 is involved in asthma and/or BHR. Further

studies to elucidate the functional role of ADAM33 are needed for a better understanding of the pathogenesis of asthma, which will in turn lead to new therapies for asthmatic patients.

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