

Original Article

S2 and T2 ADAM33 polymorphisms and haplotype associated with asthma in the Zhuang population in Guangxi, China

Siqiao Liang, Jingmin Deng, Xuan Wei, Zhangrong Chen, Chen Gong, Meiling Yang, Zixiu Wang

Department of Respiratory Medicine, The First Affiliated Hospital of Guangxi Medical University, Nanning, China

Received July 24, 2016; Accepted September 18, 2016; Epub December 15, 2016; Published December 30, 2016

Abstract: Objective: Discovered through positional cloning, a disintegrin and metalloproteinase domain-containing protein 33 (ADAM33) represents a novel gene that may increase asthma risk in different ethnic groups. This study was conducted to determine whether the single nucleotide polymorphisms (SNPs) in ADAM33 are associated with asthma risk in the large population of ethnic minorities in Guangxi Zhuang, China. Methods: A total of 226 subjects were involved in the study, including 119 normal controls and 107 asthmatic patients. Polymerase chain reaction-restriction fragment length polymorphism was used to genotype ADAM33 polymorphisms S2 and T2. Chi-square test and logistic regression model were used to analyze the data using SPSS version 16.0 and SHEsis. Results: The allele and genotype frequencies of S2 SNP statistically varied between the asthmatic cases and controls. S2 SNP was determined to be statistically associated with asthma in allele comparison (OR: 0.423, 95% CI: 0.273-0.654, $P < 0.001$), heterozygote genotype comparison (OR: 0.527, 95% CI: 0.302-0.920, $P = 0.024$), and dominant model comparison (OR: 0.421, 95% CI: 0.244-0.724, $P = 0.002$). The CA and GG haplotypes of S2 and T2 in the control groups were higher than those in the asthmatic cases with statistical significance, whereas the haplotype CG was significantly higher in the latter group. Conclusion: S2 (C>G) polymorphisms may reduce the risks of asthma among the large population of Guangxi Zhuang.

Keywords: ADAM33, asthma, polymorphism, Zhuang population

Introduction

Asthma is a complex and common respiratory disease characterized by airway inflammation, bronchial hyperresponsiveness, and airflow obstruction. Long-term repeated episodes of this chronic inflammatory disease may lead to airway remodeling and eventually cause irreversible airflow limitation in the respiratory system. Over 300 million people worldwide suffer from asthma, which continuously prevails, especially in developing countries [1]. The exact mechanism of this respiratory condition is yet to be clearly understood though epidemiological studies have specified that many factors, including environmental, immunological, and multiple genetic factors, influence its development. A recently proposed perspective asserts that asthma has significant genetic components, with heritability estimates varying between 35% and 95% [2]. Many studies have

investigated the gene variants that contribute to asthma pathogenesis. ADAM33, a disintegrin and metalloproteinase domain-containing protein 33 has been extensively studied in this context.

Through a genome-wide scan on 460 Caucasian affected sib-pair families in 2002, ADAM33 located on chromosome 20p13 was determined linked to asthma and bronchial hyperresponsiveness [3]. ADAM33, which encodes a pass integral membrane glycoprotein, is a member of the multifunctional ADAM family, a subfamily of transmembrane metalloproteinases that includes both a disintegrin and metalloproteinase domain [3]. Based on the character of these domains, ADAM33 is assumed to play a major role in cell-cell and cell-matrix interactions, cell fusion, cell adhesion, and cell signaling. The expression of ADAM33 in the airway is largely restricted to mesenchymal cells, includ-

ADAM33 polymorphisms associated with asthma

Table 1. The primers, restriction enzymes and length of digested fragments of the two SNPs for PCR-RLFP genotyping

Polymorphisms	Location	Alleles	Primers for PCR	Annealing temp. °C	Restriction enzyme	Digested fragments (bp)
S2 rs528557	Exon 19	C>G	Forward: 5-GGGGAACCGCAGGAGTA-3 Reverse: 5-TGAGAGCCCGAGGAGTG-3	62	BanI	324/135
T2 rs2280090	Exon 20	G>A	Forward: 5-TTCTCAGGGTCTGGGAGAAA-3 Reverse: 5-GCCAACCTCCTGGACTCTTA-3	55	HpyCH4III	200/110

ing fibroblasts and smooth muscle cells. Several studies have posited that the levels of *ADAM33* mRNA and protein expression in bronchoalveolar lavage fluid or bronchial biopsies are higher in asthmatic patients than in healthy controls [4, 5]. This finding is the basis for the assumption that *ADAM33* plays an important role in airway remodeling and in the development and progression of asthma.

ADAM33 is a highly polymorphic locus that contains over 70 single nucleotide polymorphisms (SNPs) [3, 6]. Recent studies report that several SNPs and/or haplotypes of *ADAM33* are significantly associated with asthma in various ethnic populations, including Caucasian, Asian, and Chinese [7-9]. Both genetic and environmental factors influence the pathogenesis of asthma; hence, the susceptibility genes of this respiratory condition may vary in different ethnic populations. For instance, Jie Z *et al.* [9] and Qu S *et al.* [10] identified that among Chinese, the T1 variant in *ADAM33* is significantly associated with asthma. By contrast, Bijanzadeh M *et al.* [11] found no significance for the *ADAM33* T1 polymorphism between asthmatic patients and controls in an Indian population. Most studies that look into the association of genetic polymorphisms with asthma in the Chinese context are focused on the Han population. Correspondingly, no study has investigated the associations of SNPs S2 (rs528557, C>G) and T2 (rs2280090, G>A) with asthma in the Zhuang population of Guangxi, China. With a population of approximately 15, 200, 000, this ethnic minority resides in south China. The Zhuang people rarely intermarry with other ethnic groups, and their lifestyle is consistent. These conditions are the reason why such ethnic group is a significant population for study. In this case-control study, we aim to examine the association of the polymorphisms and haplotypic of *ADAM33* S2 and T2 with asthma in the Zhuang population of China.

Materials and methods

Subjects

A total of 107 asthmatic patients and 119 healthy controls were included in the study. The diagnosis of asthma was achieved in accordance with the guidelines of the Global Initiative for Asthma (2008). The controls have no history of asthma, rhinitis, or other allergic or lung diseases. Both groups were recruited for the study between February 2010 and April 2013 in the Department of the First Affiliated Hospital of Guangxi Medical University, Guangxi, China. All study subjects were unrelated members of the Chinese Zhuang population, and permanently resided in Guangxi (three generations or more). Each respondent submitted a written informed consent to participate in the research. The work was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (Approval Number: 2016 KY-E-027).

DNA extraction and genotyping

Genome extraction was accomplished using a DNA extraction kit following the manufacturer's instructions (Tiangen, Shanghai, China) with 2 ml of peripheral blood. The presence of two susceptibility SNPs for asthma (i.e., S2 and T2) was analyzed. The primers were obtained from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP) was performed to genotype the SNPs. The 25 µL of PCR reaction consisted of 2 µL of genomic DNA, 12.5 µL of 2×Master Mix (Fermentas, Canada), 8.5 µL of ddH₂O, and 1 µL of each primer. The PCR products were digested overnight with restriction enzymes (Fermentas, Canada) according to the manufacturer's protocol and then analyzed with 2% of agarose gel electro-

ADAM33 polymorphisms associated with asthma

Table 2. The genotype and allele frequencies of the two SNPs

SNP	Genotype	Case (n=107)	Control (n=119)	χ^2 value	P value
S2	CC	66 (61.7%)	48 (40.3%)	18.87	0
	CG	41 (38.3%)	57 (47.9%)		
	GG	0 (0%)	14 (11.8.4%)		
T2	AA	0 (0%)	3 (2.5%)	2.89	0.259
	AG	24 (22.4%)	31 (26.1%)		
	GG	83 (77.6%)	85 (71.4%)		

Table 3. The contribution of ADAM33 gene polymorphisms to the risk of asthma in different comparisons

Polymorphisms	Comparisons	OR	95% CI	P value
S2 rs528557	G/C	0.423	0.273-0.654	<0.001
	GG/CC	-	-	0.998
	GC/CC	0.527	0.302-0.920	0.024
	GG+GC/CC	0.421	0.244-0.724	0.002
	GG/GC+CC	-	-	0.998
T2 rs2280090	A/G	0.711	0.408-1.240	0.229
	AA/GG	-	-	0.999
	AG/GG	0.806	0.434-1.497	0.495
	AA+AG/GG	0.744	0.404-1.372	0.344
	AA/AG+GG	-	-	0.999

phoresis. All genotype results were confirmed in 5% of the samples randomly selected for sequencing. **Table 1** shows the primers, annealing temperature, restriction enzymes, and length of the digested fragments of each SNP in ADAM33 for PCR-RLFP genotyping.

Statistical analyses

The Hardy-Weinberg equilibrium (HWE) of the control group was determined through chi-square (χ^2) test, which was also conducted to calculate the differences in genotypic distribution between the cases and controls. The risk between the genetic polymorphisms and asthma was calculated by logistic regression analysis. Each SNP was analyzed through a variant allele comparison (A vs. a), homozygote genotype comparison (AA vs. aa), heterozygote genotype comparison (Aa vs. aa), dominant model comparison (AA+Aa vs. aa), and recessive model comparison (AA vs. Aa+ aa). The odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated. P value less than 0.05 (two-tailed) was considered as a difference of statistical significance. The statistical analyses were performed using SPSS 16.0, and the whole analysis was age and sex-adjusted. The

haplotype frequencies and linkage disequilibrium (LD) were calculated using SHEsis, an online program (<http://analysis.bio-x.cn/myAnalysis.php>). The inspection level was α : 0.05.

Results

Characteristics of study subjects

A total of 226 subjects were involved in the study, including 107 asthmatic patients (mean age 37.69 ± 12.23 years, 44 males and 63 females) and 119 controls (mean age 34.67 ± 11.46 years, 49 males and 70 females). No significant differences were observed in the distribution of age and gender in this case-control study ($P > 0.05$).

Genotype and allele frequencies of SNPs S2 and T2

The distributions of the two SNPs were both in HWE ($P > 0.05$) in the control group. **Table 2** shows the genotypic distributions of the SNPs. The chi-square test indicated that the distributions of the allele and genotype frequencies of S2 SNP statistically varied between the case and control groups. **Table 3** demonstrates the contributions of the genetic polymorphisms to the risk of asthma calculated by logistic regression analysis through variant allele comparison, homozygote genotype comparison, heterozygote genotype comparison, dominant model comparison, and recessive model comparison. For S2, significant associations were observed using the allele comparison (OR: 0.423, 95% CI: 0.273-0.654, $P < 0.001$), heterozygote genotype comparison (OR: 0.527, 95% CI: 0.302-0.920, P : 0.024), and dominant model comparison (OR: 0.421, 95% CI: 0.244-0.724, P : 0.002). The statistical analysis showed no significant difference for the SNP T2 in any of the comparisons between the asthmatic patients and controls.

Haplotype and LD analysis in ADAM33

The SNPs were used to construct haplotypes. In particular, four haplotypes (frequency > 0.02) were generated using SHEsis, and their frequencies are listed in **Table 4**. The frequency of

ADAM33 polymorphisms associated with asthma

Table 4. ADAM33 haplotype profiles in the study population

Haplotype	Haplotype frequency		P value	OR	(95% CI)
	Case (freq)	Control (freq)			
Hap1 C A*	2.25 (0.011)	11.83 (0.050)	0.017	0.204	0.048-0.857
Hap2 C G*	170.75 (0.798)	141.17 (0.593)	<0.001	2.707	1.775-4.129
Hap3 G A*	21.75 (0.102)	25.17 (0.106)	0.885	0.956	0.522-1.753
Hap4 G G*	19.25 (0.090)	59.83 (0.251)	<0.001	0.294	0.170-0.512

haplotype CG was significantly higher in the asthma group than that in the controls, but those of haplotypes CA and GG were low in the asthma group. No significant difference was noted between the two groups for the GA haplotype. The LD analysis showed a weak LD between the polymorphisms (D' : 0.690, r^2 : 0.192).

Discussion

Asthma, which is characterized by chronic airway inflammation that leads to bronchial hyper-responsiveness and reversible airway obstruction, is among the most prevalent chronic diseases worldwide. A recent study shows that the absolute number of prevalent asthmatic cases will continuously rise, and such respiratory condition will remain as a major burden among individuals and the health care system [12]. Environmental and genetic factors are both involved in the etiology of asthma. As such, the genetic risk factors related to this respiratory disease should be identified to help diagnose its subtypes and implement additional satisfactory therapeutic choices. The genetic studies of asthma in the Zhuang population of China have only been initiated recently, and limited information is available. To our knowledge, this study is the first to report about the relationship between ADAM33 polymorphisms and asthma in the Guangxi Zhuang population.

In this study, we analyzed two polymorphisms (i.e., S2 and T2) of ADAM33 to investigate their association with asthma. The ADAM33 S2 and 3 SNP haplotypes were determined associated with asthma in the Guangxi Zhuang population of China. A statistical association was observed for the S2 SNP by variant allele comparison, heterozygote genotype comparison, and dominant model comparison. A single copy of the mutant G allele (S2) may decrease the risk of asthma or act as a resistance allele for asthma in the studied population. Contrarily, T2 SNP

was determined unrelated to asthma similar to rs2280090, G>A mutation. The CA and GG haplotypes of S2 and T2 were slightly lower in the asthmatic group than in the controls. This finding indicates that haplotypes CA and GG may decrease the asthma risk

in the current study population. The haplotype CG was significantly higher in the asthma patients than in controls, implying that it may increase the risk of asthma in the population. A peculiar distribution of haplotypes may carry important information about the unknown causal variants that may be peculiar to a region. All these findings can improve one's understanding of the underlying mechanisms of asthma and may help provide further diagnostic and prognostic information for asthmatic patients. In addition, these results can point to a new strategy in genetic therapy for adult asthmatic patients.

Previous studies have shown that the association between ADAM33 polymorphisms and asthma varies in people of different ethnic origins. For the S2 SNP, a significant association was observed in the African American population in Europe, Pakistani and Jordanian [7, 13, 14], whereas no significant difference was observed between the asthmatic patients and controls in Czech Republic [15]. Tripathi P determined that the CC and CG genotypes as well as the C allele frequency of the S2 SNP located in ADAM33 are significantly associated with the increased risk of asthma [16] in an Indian population. This finding is consistent with ours. However, Hirota T discovered that in the Japanese context, the GG genotype and G allele frequencies of S2 SNP are significantly associated with the increased risk of asthma [17]. In the Han Chinese population, Chi X and Fan JG found that the G allele of S2 SNP is more common in the asthma patients than in controls [18, 19]. This observation suggests that an increased risk of asthma is associated with a G allele in the Shandong Han population of China. By contrast, Jie Z proved that the S2 SNP of ADAM33 is unassociated with asthma in the Shanghai Han population of China [9]. For T2 SNP, significant associations have been noted in the East China Han population [9], Northeast China Han population [20], the Japanese popu-

lation [17], and so on. All these results imply that A allele mutation can increase asthma risk. However, several previous studies showed that T2 SNP is not associated with asthma in the populations of northern Chinese [10], Shandong Han Chinese [18], Chinese Uyghur [21], Iranian [22], Pakistani [14] or German [23].

The differences in observations may be explained by various conditions. First, a single study may have a small sample size, which may have resulted in low statistical power. Second, gene-environment interactions may vary in different ethnicities. In other words, different genetic backgrounds and different environmental exposures may affect the individuals' sensitivity to asthma. Even in the same ethnic Han population, considerable diversity in genetic background as well as social and cultural habits can be observed between subgroups. Therefore, the relationship between gene polymorphism and a certain disease in different ethnic populations should be analyzed with a large sample size.

Asthma involves the interaction of genetic and environmental components that contribute to its progression. Recent years have seen rapid progress in mapping novel asthma loci through genome-wide association studies such as 17q21 harboring *ORMDL3* [24]. However, previously identified asthma susceptibility genes, such as *ADAM33*, were found by positional cloning [3]. *ADAM33* was determined associated with airway remodeling, bronchial hyperresponsiveness, and asthma in several diverse populations [3]. In a cohort study on Dutch asthmatic patients followed for 20 years, *ADAM33* SNPs were considered associated with the accelerated exacerbation of asthma [25]. Both S2 and T2 SNPs are located in the exons of *ADAM33*; hence, the amino acids change with alterations in the bases, eventually changing the protein translation. In addition, S2 and T2 are located in the cytoplasmic domain of *ADAM33* in which the changes in the alleles of S2 and T2 SNPs can potentially alter intracellular signaling, and thereby causing increased fibroblast and smooth muscle proliferation that can lead to airway remodeling.

Our research findings generally indicated that S2 (C→G) polymorphisms may reduce the risks of asthma in the Guangxi Zhuang population.

These observations may help detect individuals with high risk of asthma and correspondingly provide timely treatments. Considering that the limited sample size in our study may produce relative risk estimates with inadequate precision, our findings should be verified by future research with an improved large-scale design in different ethnic populations.

Acknowledgements

This work is supported by the Scientific and Technological Research Project of Guangxi College and University (Grant no. YB2014068), Guangxi Medical University Science Foundation for Youths (Grant no. GXMUYSF2014033). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Jingmin Deng, Department of Respiratory Medicine, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi, China. Tel: +86 0771-5356702; Fax: +86 0771-5356702; E-mail: ldy666@163.com

References

- [1] Barnes PJ. New therapies for asthma: is there any progress? *Trends Pharmacol Sci* 2010; 31: 335-343.
- [2] Ober C and Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011; 242: 10-30.
- [3] Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, Torrey D, Pandit S, McKenny J, Braunschweiger K, Walsh A, Liu Z, Hayward B, Folz C, Manning SP, Bawa A, Saracino L, Thackston M, Benčekroun Y, Capparell N, Wang M, Adair R, Feng Y, Dubois J, FitzGerald MG, Huang H, Gibson R, Allen KM, Pedan A, Danzig MR, Umland SP, Egan RW, Cuss FM, Rorke S, Clough JB, Holloway JW, Holgate ST and Keith TP. Association of the *ADAM33* gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418: 426-430.
- [4] Ito I, Laporte JD, Fiset PO, Asai K, Yamauchi Y, Martin JG and Hamid Q. Downregulation of a disintegrin and metalloproteinase 33 by IFN- γ in human airway smooth muscle cells. *J Allergy Clin Immunol* 2007; 119: 89-97.

ADAM33 polymorphisms associated with asthma

- [5] Foley SC, Mogas AK, Olivenstein R, Fiset PO, Chakir J, Bourbeau J, Ernst P, Lemiere C, Martin JG and Hamid Q. Increased expression of ADAM33 and ADAM8 with disease progression in asthma. *J Allergy Clin Immunol* 2007; 119: 863-871.
- [6] Chae SC, Yoon KH and Chung HT. Identification of novel polymorphisms in the Adam33 gene. *J Hum Genet* 2003; 48: 278-281.
- [7] Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL, Xu J, Bleeker ER and Meyers DA. Association of a disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. *J Allergy Clin Immunol* 2003; 112: 717-722.
- [8] Tripathi P, Awasthi S, Prasad R and Ganesh S. Haplotypic association of ADAM33 (T+1, S+1 and V-3) gene variants in genetic susceptibility to asthma in Indian population. *Ann Hum Biol* 2012; 39: 479-483.
- [9] Jie Z, Hu Z, Bai C and Jin M. ADAM33 gene polymorphisms associate with asthma susceptibility and severity in East China han population. *J Asthma* 2011; 48: 979-985.
- [10] Qu S, Sun D, Wang Y, Zhang C, Lv Y and Yao L. Association of ADAM33 polymorphisms with childhood asthma in a northern Chinese population. *Exp Mol Pathol* 2011; 91: 775-779.
- [11] Bijanzadeh M, Ramachandra NB, Mahesh PA, Mysore RS, Kumar P, Manjunath BS and Jayaraj BS. Association of IL-4 and ADAM33 gene polymorphisms with asthma in an Indian population. *Lung* 2010; 188: 415-422.
- [12] To T, Stanojevic S, Feldman R, Moineddin R, Atenafu EG, Guan J and Gershon AS. Is asthma a vanishing disease? A study to forecast the burden of asthma in 2022. *BMC Public Health* 2013; 13: 254.
- [13] Zihlif M, Zihlif N, Obeidat NM, Mahafza T, Froukh T, Ghanim MT, Al-Akhras FM and Naffa R. Association between ADAM33 polymorphisms and susceptibility with adult and childhood asthma among Jordanians. *Genet Test Mol Biomarkers* 2014; 18: 767-774.
- [14] Sabar MF, Ghani MU, Shahid M, Sumrin A, Ali A, Akram M, Tariq MA and Bano I. Genetic variants of ADAM33 are associated with asthma susceptibility in the Punjabi population of Pakistan. *J Asthma* 2016; 53: 341-348.
- [15] Godava M, Kopriva F, Bohmova J, Vodicka R, Dusek L, Cvanova M, Muzik J, Markova M, Schneiderova E and Vrtel R. Association of STAT6 and ADAM33 single nucleotide polymorphisms with asthma bronchiale and IgE level and its possible epigenetic background. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2012; 156: 236-247.
- [16] Tripathi P, Awasthi S, Prasad R, Husain N and Ganesh S. Association of ADAM33 gene polymorphisms with adult-onset asthma and its severity in an Indian adult population. *J Genet* 2011; 90: 265-273.
- [17] Hirota T, Hasegawa K, Obara K, Matsuda A, Akahoshi M, Nakashima K, Shirakawa T, Doi S, Fujita K, Suzuki Y, Nakamura Y and Tamari M. Association between ADAM33 polymorphisms and adult asthma in the Japanese population. *Clin Exp Allergy* 2006; 36: 884-891.
- [18] Chi X, Wang L, Wang J, Li Q, Wang X and Xiao W. Association of ADAM33 gene polymorphisms with asthma in a Chinese population. *Clin Respir J* 2013; 7: 16-20.
- [19] Fan JG, Wang ZA and Zhao HX. The ADAM33 S2 polymorphism is associated with susceptibility to pediatric asthma in the Chinese Han population. *Genet Test Mol Biomarkers* 2015; 19: 573-578.
- [20] Su D, Zhang X, Sui H, Lu F, Jin L and Zhang J. Association of ADAM33 gene polymorphisms with adult allergic asthma and rhinitis in a Chinese Han population. *BMC Med Genet* 2008; 9: 82.
- [21] Yilihamu N, Wushouer Q, Arkin K, Xin H and Yadav U. Association of a disintegrin and metalloprotease 33 gene polymorphisms with asthma. *Mol Clin Oncol* 2014; 2: 1076-1080.
- [22] Karimi MR, Faridhosseini R, Abbaszadegan MR, Azad FJ, Shirkani A, Riyahi A, Montazar M and Gholamin M. Association of ADAM33 gene polymorphisms with allergic asthma. *Iran J Basic Med Sci* 2014; 17: 716-721.
- [23] Schedel M, Depner M, Schoen C, Weiland SK, Vogelberg C, Niggemann B, Lau S, Illig T, Klopp N, Wahn U, von Mutius E, Nickel R and Kabesch M. The role of polymorphisms in ADAM33, a disintegrin and metalloprotease 33, in childhood asthma and lung function in two German populations. *Respir Res* 2006; 7: 91.
- [24] Michel S, Liang L, Depner M, Klopp N, Ruether A, Kumar A, Schedel M, Vogelberg C, von Mutius E, von Berg A, Bufe A, Rietschel E, Heinzmann A, Laub O, Simma B, Frischer T, Genuneit J, Gut IG, Schreiber S, Lathrop M, Illig T and Kabesch M. Unifying candidate gene and GWAS Approaches in Asthma. *PLoS One* 2010; 5: e13894.
- [25] Jongepier H, Boezen HM, Dijkstra A, Howard TD, Vonk JM, Koppelman GH, Zheng SL, Meyers DA, Bleeker ER and Postma DS. Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma. *Clin Exp Allergy* 2004; 34: 757-760.