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ADAM33 and ADAM12 genetic polymorphisms and their expression in Egyptian children with asthma

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ABSTRACT

Background: The ADAM family is involved in some pathologic processes, such as inflammation and asthma. **Objectives:** To assess the association between *ADAM33* and *ADAM12* single-nucleotide polymorphisms (SNPs) with asthma risk and severity and to investigate the effect of *ADAM33* and *ADAM12* polymorphisms on expression of these proteases in sputum.

Methods: Two SNPs of the *ADAM33* gene, F+1 (rs511898) G/A and ST+4 (rs44707) A/C, and 2 SNPs of the *ADAM12* gene, rs3740199 and rs1871054, were analyzed in 400 asthma cases and 200 controls aged 3 to 14 years using the polymerase chain reaction–restriction fragment length polymorphism method. Messenger RNA expression profile of *ADAM33* and *ADAM12* proteases in sputum from studied groups was determined by reverse transcription polymerase chain reaction.

Results: *ADAM33* F+1 homozygous mutant genotype (AA) and ST+4 heterozygous and homozygous mutant genotype (AC and CC) and mutant alleles of both polymorphisms were significantly associated with asthma risk and severity in moderate and severe subgroups. Patients with the *ADAM12* (rs3740199) CC genotype were at increased risk for moderate and severe asthma. Messenger RNA levels of *ADAM12* were significantly increased in asthmatic children compared with controls, whereas we were not able to detect the expression of *ADAM33* in the sputum of the groups studied. The *ADAM12* expression was significantly higher in homozygous CC (variant type) compared with homozygous GG (wild type) of both *ADAM12* rs3740199 and rs1871054 in the asthmatic group.

Conclusion: Our analysis suggests a likely role for *ADAM33* and *ADAM12* in the development of asthma in Egyptian children. Furthermore, *ADAM12* polymorphisms may affect *ADAM12* expression in asthma.

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Introduction

The prevalence of childhood asthma has increased worldwide in recent decades.¹ It is the most frequent chronic respiratory disease during childhood. Asthma exacerbation is one of the main causes of child morbidity and hospitalization.² Asthma is often associated with structural remodeling of the airways and is characterized by airway epithelial damage, wall thickening, and subepithelial fibrosis.³ The cause of this disease is believed to be a complex combination of multiple genetic and environmental factors that lead to heterogeneous phenotypes, such as variable degrees of atopic involvement and severity.⁴ Early family and twin studies support the role of genetics in the development of the disease.⁵

The ADAM family is a subgroup of the metzincin metalloproteinase superfamily and is involved in physiologic processes, such as fertilization, cell fusion, cell migration, cytokine and growth factor shedding, muscle development, and immune response.^{6,7} On the other hand, it is involved in some pathologic processes, such as inflammation and cancer.⁸ The first positional-cloned asthma susceptibility gene is a member of the ADAM family, *ADAM33*, which is located on the human chromosome 20p13. The *ADAM33* gene is highly polymorphic, containing more than 70 single-nucleotide polymorphisms (SNPs) with extensive linkage disequilibrium.⁹ *ADAM33* might be associated with childhood asthma, lung function decline, bronchial hyper-responsiveness, and airway remodeling because of its high expression in lung fibroblasts and bronchial smooth muscle.^{10,11} It also protects the airway from increased repair processes.¹²

Another member of the ADAM gene family is *ADAM12*, which is located on chromosome 10q26. It has 2 alternatively spliced transcripts: a shorter secreted form (ADAM-12S) and a longer

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membrane-bound form (ADAM-12L). The shorter form is found to stimulate myogenesis.¹³ ADAM12 supports cell attachment through its interaction with integrins ($\alpha 3$, $\alpha 4$, and $\alpha 5$) and with syndecan 4. Moreover, ADAM12 is implicated in the transactivation of epidermal growth factor receptor through the shedding of ligands such as heparin-binding epidermal growth factor.^{14,15}

Many studies have found that the ADAM33 polymorphisms confer susceptibility to asthma in different groups from the United Kingdom, Thailand, Germany, India, and Japan.^{16–19} However, no study has found an association of SNPs F+1 (rs511898) and ST+4 (rs44707) with asthma risk and severity in Egyptian children. In addition, the association between asthma and the ADAM12 gene polymorphism has not been yet studied.

In the present study, ADAM33 (F+1 and ST+4) and ADAM12 (rs3740199 and rs1871054) SNPs were selected and detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The associations of the SNPs with asthma risk and severity were analyzed. We also estimated the messenger RNA (mRNA) expression profile of ADAM33 and ADAM12 proteases in sputum from the studied groups.

Methods

Participants

This case-control study comprised 400 asthmatic children and 200 healthy controls. Following the American Thoracic Society guidelines, asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months and a greater than 12% reversibility of forced expiratory volume in 1 second (FEV₁) spontaneously or after β_2 -agonist inhalation. Allergic and nonallergic asthma was based on a questionnaire completed separately for each child by the parents. The questionnaire included demographics, medical history, skin prick test data, and medicines taken during the last 12 months. The diagnosis and classification of the clinical severity of asthma were made according to the Global Initiative for Asthma guidelines.²⁰ The asthmatic children were categorized into severity groups by using levels of FEV₁ percent predicted. The patients were classified as having mild (FEV₁, >80%), moderate (FEV₁, 60%–80%), or severe (FEV₁, <60%). Patients using concomitant antiasthma or antiallergy medication for at least 3 weeks before the clinical evaluation were excluded. The control group consisted of 200 children presented to the Pediatric Department of Zagazig University Hospital with concerns other than respiratory or atopic diseases. This study was approved by the institutional ethics committee. Written informed consent for participation was obtained from parents or guardians of all participants. Characteristics of the participants are presented in Table 1.

Blood Sample Collection

Five milliliters of venous blood obtained from each participant were collected in EDTA-containing tubes. Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin).

Sputum Collection and RNA Extraction

We collected the sputum from 54 asthmatic children and 12 controls. Sputum processing was performed as previously reported.^{21,22} The patients were asked to rinse their mouths and gargle with water before sputum collection. Samples were collected in a sterile cup. Sputum can be collected from even very young children by inducing cough and sputum production with nebulized saline or bronchodilators and then asking the patient to expectorate the sputum or suctioning the back of the pharynx to

Table 1

Clinical profile of the study participants

Variable	Patients (n = 400)	Controls (n = 200)	P value
Age, y			.29 ^a
Mean (SD)	8.5 (3.6)	8.8 (2.6)	
Range	3–14	4–13	
Sex, No. (%)			.58 ^a
Male	215 (53.8)	102 (51.0)	
Female	185 (46.3)	98 (49.0)	
Mean age at onset, y	4.3		
Residence			.79 ^a
Urban	228 (57.0)	117 (58.5)	
Rural	172 (43.0)	83 (41.5)	
Family history of asthma			<.001 ^a
Negative	133 (33.3)	167 (83.5)	
Positive	267 (66.8)	33 (16.5)	
FEV ₁ , %	88.3 (17)	97.3 (18)	.004 ^a
Skin prick test			<.001 ^a
Negative	192 (48.0)	200 (100)	
Positive	208 (52.0)	0	
Disease severity			<.001 ^b
Mild	193 (48.3)		
Moderate	122 (30.5)		
Severe	85 (21.3)		

Abbreviation: FEV₁, forced expiratory volume in 1 second.

^aBetween patients and controls.

^bBetween the disease severity categories.

collect sputum. After induction, all sputum microscopically free of salivary contamination was selected for processing. The samples were kept at 4°C. Then samples were diluted with an equal volume of phosphate-buffered saline containing 10 mM dithiothreitol (Calbiochem, San Diego, California) and centrifuged at 400g for 10 minutes at 4°C to separate cellular and fluid phases. After centrifugation and removal of the supernatant fluid, the sputum cell pellets were resuspended in phosphate-buffered saline to a final volume of 2 mL, followed by filtration through a gauze (pore size, 1 mm) to remove clumps. Total RNA was isolated from the cell suspension using an RNeasy Mini kit, including DNase I digestion, following the manufacturer's protocol (Qiagen, Hilden, Germany). The ratio of absorbance values at 260 and 280 nm indicates an estimate of RNA purity.

Genotyping of ADAM33 and ADAM12

Two SNPs of the ADAM33 that have an allelic association with asthma were selected for screening: F+1 and ST+4. Genotyping was performed by PCR-RFLP.²³ The polymorphisms of the ADAM12 gene (rs3740199 and rs1871054) were also amplified by PCR and analyzed by RFLP.²⁴ Amplification was performed using a PTC-100 thermal cycler (MJ Research Inc, Watertown, Massachusetts). The PCR reaction mixtures of the total volume of 25 μ L included 10 μ g of genomic DNA, 5 pmol of each primer (Promega, Madison, WI), and 1 $\times$ PCR mix (Taq PCR Master Mix Kit; Qiagen) containing 200 μ mol/L of each deoxynucleotide triphosphate, 5 μ L of 10 $\times$ reaction buffer, 1.25 U of Taq gold polymerase, and 4 mmol/L magnesium chloride. The PCR products were then digested overnight by 1 U of restriction endonuclease (Fermentas, Waltham, Massachusetts) at 37°C and were thereafter separated by 1.5% agarose gel electrophoresis. Detailed information about the location of SNPs, primer sequences, and restriction enzymes is summarized in Table 2.

ADAM33 and ADAM12 mRNA Expression Levels

Expression levels of ADAM33 and ADAM12 mRNAs were determined by semiquantitative RT-PCR according to Paulissen et al²⁵ with some modifications. RNA extracted from the sputum was reverse transcribed with a QuantiTect Reverse Transcription Kit (Qiagen). The used primers were as follows: forward:

Table 2Primers and PCR conditions for *ADAM33* and *ADAM12* PCR-RFLP genotyping

Polymorphic site	Primer sequence	PCR conditions	Restriction enzyme and fragment size, bp
<i>ADAM33</i> F+1	Forward: 5'-GTATCTATAGCCCTCCAAATCAGAAGGCC-3' Reverse: 5'-GGACCCTGAGTGAAGCTG-3'	40 Cycles: 45 seconds at 95°C, 45 seconds at 60°C, 30 seconds at 72°C	<i>MspI</i> A 166 G 137+29
<i>ADAM33</i> ST+4	Forward: 5'-CACTTCTCTGCACAAATCACCTCTGTCGTC-3' Reverse: 5'-GAGCACTCCCAAGACCAGGCTATGTCAG-3'	40 Cycles: 45 seconds at 95°C, 45 seconds at 64°C, 30 seconds at 72°C	<i>MjaI</i> V C 277 A 246+31
<i>ADAM12</i> rs3740199 C > G	Forward: 5'-TCT GCT TTG ACA GTG TGC ATG GCT-3' Reverse: 5'-GCT CTC CAG AGT AGC ACA GGT CAC-3'	37 Cycles: 1 minute at 95°C, 3 minutes at 60°C, 1 minutes at 72°C	<i>Ppil</i> C 231+34 G 265
<i>ADAM12</i> rs1871054 C > T	Forward: 5'-TCT GCT TTG ACA GTG TGC ATG GCT-3' Reverse: 5'-GCT CTC CAG AGT AGC ACA GGT CAC-3'	37 Cycles: 1 minute at 95°C, 3 minutes at 62°C, 1 minute at 72°C	<i>Eco91I</i> C 131+19 T 150

Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

5'-ATGAGTGGCCTGATCACCTCA-3' and reverse: 5'-TGTTTCAA GTTTCGG TGCCGAG-3' for *ADAM33*, forward: 5'-GGAATTGTCATG GACCATTCAG-3' and reverse: 5'-TTCCTGCTGCAACTG CTGAACA-3' for *ADAM12*, and forward: 5'-GTTCACCCACTAATAGGGAACGTGA-3' and reverse: 5'-GATTCTGACTTAGAGGCGTTCACT-3' for 28S ribosomal RNA (housekeeping control gene). Cycle parameters were 94°C for 10 minutes (hot start) followed by 37 cycles (94°C for 15 seconds, 68°C for 20 seconds, and 72°C for 10 seconds) and 72°C for 2 minutes to finish. All PCR products were subjected to electrophoresis using 1.5% agarose gel, stained with ethidium bromide. The product was visualized using gel documentation system. Relative expression of each gene was calculated as densitometric units of each studied gene divided by densitometric units of 28S.

Statistical Analysis

Numerical values were analyzed by analysis of variance and *t* test. Proportions of groups were compared by the χ^2 test. In addition, the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated as a measure of the association of the *ADAM33* and *ADAM12* genotypes and alleles with groups. Genotype frequencies in cases and controls were tested for Hardy-Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the χ^2 test. The appropriate sample size and power of the study were determined using PAWE-3D. PAWE-3D calculations revealed that the sample size, together with the specified study design, allele frequencies, and allowable error rates, can give as high as 85% power and can detect the variant allele frequency of at least 0.05 and genotype relative risk of 1.8 or greater at 80% power. When we applied Bonferroni corrections to correct for multiple testing and to determine whether the observed associations were false positive, we observed that empirical probability values remained significant for the associations of the *ADAM33* ST+4 and F+1 and *ADAM12* (rs3740199) with asthma risk. The threshold for significance was $P < .05$.

Results

Associations of *ADAM33* and *ADAM12* Gene Polymorphisms With Asthma Risk

The genotype and allele frequencies of the *ADAM33* and *ADAM12* gene polymorphisms in asthmatic children and controls are given in Table 3 and Figure 1. Genotype frequencies of all polymorphisms were in agreement with Hardy-Weinberg equilibrium in each study group. Our results indicate that the SNPs at F+1 and ST+4 of

ADAM33 and *ADAM12* (rs3740199) were associated with an increased risk of asthma. Compared with the F+1 GG genotype carriers, increased susceptibility to asthma for the AA carriers was detected (OR, 3.1; 95% CI, 1.9–5.2; $P < .001$). Compared with the ST+4 AA genotype carriers, increased susceptibilities to asthma for the AC and CC carriers were detected (OR, 1.9; 95% CI, 1.3–2.7; $P = .002$; and OR, 2.6; 95% CI, 1.6–4.4; $P < .001$, respectively). Moreover, increased risk of asthma was observed with the homozygous mutant genotype CC of *ADAM12* (rs3740199) (OR, 2.6; 95% CI, 1.5–4.3; $P < .001$). No significant association was detected between the *ADAM12* (rs1871054) and asthma risk.

Table 3Genotype and allele frequencies of *ADAM33* and *ADAM12* polymorphisms in studied groups

Genotype and Allele	Control group, No. (%)	Asthma group, No. (%)	OR (95% CI)	<i>P</i> value ^a
<i>ADAM33</i> (F+1)				
Genotype				
GG	58 (29.0)	77 (19.25)	1 [Reference]	
GA	107 (53.5)	178 (44.5)	1.3 (0.8–1.9)	.34
AA	35 (17.5)	145 (36.25)	3.1 (1.9–5.2)	<.001
Allele				
G	223 (55.8)	332 (41.5)	1 [Reference]	
A	177 (44.2)	468 (58.5)	1.8 (1.4–2.3)	<.001
<i>ADAM33</i> (ST+4)				
Genotype				
AA	87 (43.5)	109 (27.2)	1 [Reference]	
AC	84 (42.0)	195 (48.8)	1.9 (1.3–2.7)	.002
CC	29 (14.5)	96 (24.0)	2.6 (1.6–4.4)	<.001
Allele				
A	258 (64.5)	413 (51.6)	1 [Reference]	
C	142 (35.5)	387 (48.4)	1.7 (1.3–2.2)	<.001
<i>ADAM12</i> (rs3740199)				
Genotype				
GG	83 (41.5)	124 (31.0)	1 [Reference]	
GC	92 (46.0)	180 (45.0)	1.3 (0.9–1.9)	.16
CC	25 (12.5)	96 (24.0)	2.6 (1.5–4.3)	<.001
Allele				
G	258 (64.5)	428 (53.5)	1 [Reference]	
C	142 (35.5)	372 (46.5)	1.6 (1.2–2)	<.001
<i>ADAM12</i> (rs1871054)				
Genotype				
GG	56 (28.0)	108 (27.0)	1 [Reference]	
GC	96 (48.0)	214 (53.5)	1.2 (0.7–1.7)	.48
CC	48 (24.0)	78 (19.5)	0.8 (0.5–1.4)	.49
Allele				
G	208 (52.0)	430 (53.75)	1 [Reference]	
C	192 (48.0)	370 (46.25)	1.2 (0.9–1.5)	.19

Abbreviations: CI, confidence interval; OR, odds ratio.

^aCompared with the control group.

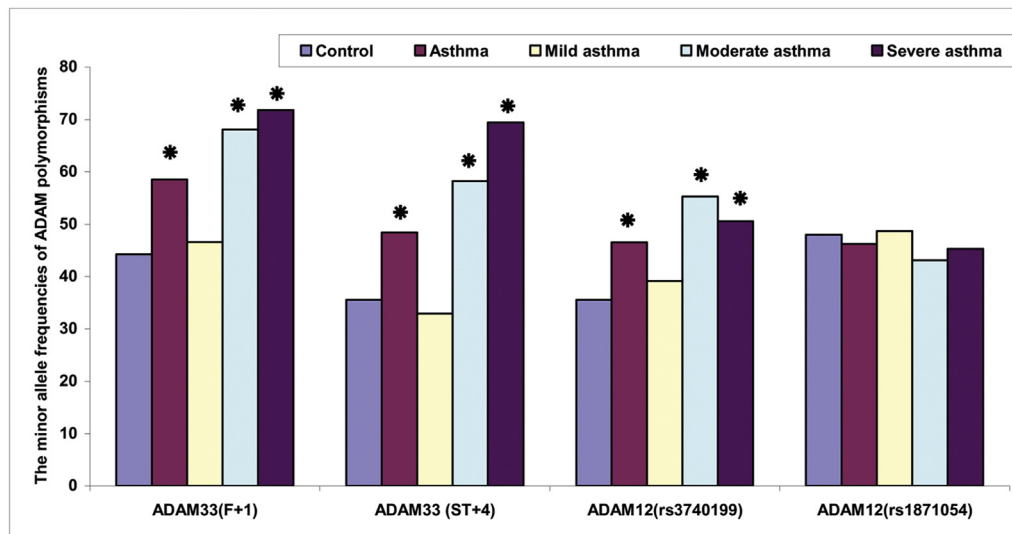


Figure 1. The minor allele frequencies for *ADAM33* and *ADAM12* polymorphisms among studied groups. Asterisk indicates $P < .05$ when compared with the control group.

Association of *ADAM33* and *ADAM12* Gene Polymorphisms With Severity of Asthma

The genotype and allele frequencies of *ADAM33* and *ADAM12* gene polymorphisms in subgroups of asthmatic patients and in healthy controls are given in Table 4 and Figure 1. Individuals with the homozygous mutant genotype (AA) of F+1 SNP, the heterozygous and homozygous mutant genotype (AC and CC) of ST+4 SNP, and the homozygous mutant genotype (CC) of *ADAM12* (rs3740199)

were at increased risk for moderate and severe asthma. *ADAM12* (rs1871054) polymorphism was not significantly associated with asthma severity.

Expression of *ADAM33* and *ADAM12* mRNA in Studied Groups

The mean (SD) relative expression of *ADAM12* mRNA in the sputum cells was 0.478 (0.242) in the asthma group ($n = 54$) and 0.146 (0.091) in the control group. mRNA transcripts for *ADAM12*

Table 4
Association of *ADAM33* and *ADAM12* gene polymorphisms with asthma severity

Genotype and Allele	Control group (n = 200), No. (%)	Mild asthma group (n = 193), No. (%)	P value ^a	Moderate asthma group (n = 122), No. (%)	P value ^a	Severe asthma group (n = 85), No. (%)	P value ^a
<i>ADAM33</i> (F+1)							
Genotype							
GG	58 (29.0)	45 (23.3)	Reference	19 (15.6)	Reference	13 (15.3)	Reference
GA	107 (53.5)	116 (60.1)	.20	40 (32.8)	.80	22 (25.9)	.90
AA	35 (17.5)	32 (16.6)	.70	63 (51.6)	<.001	50 (58.8)	<.001
Allele							
G	223 (55.8)	206 (53.4)	Reference	78 (31.9)	Reference	48 (28.2)	Reference
A	177 (44.2)	180 (46.6)	.55	166 (68.1)	<.001	122 (71.8)	<.001
<i>ADAM33</i> (ST+4)							
Genotype							
AA	87 (43.5)	92 (47.7)	Reference	10 (8.2)	Reference	7 (8.2)	Reference
AC	84 (42.0)	75 (38.8)	.50	82 (67.2)	<.001	38 (44.7)	<.001
CC	29 (14.5)	26 (13.5)	.70	30 (24.6)	<.001	40 (47.1)	<.001
Allele							
A	258 (64.5)	259 (67.1)	Reference	102 (41.8)	Reference	52 (30.6)	Reference
C	142 (35.5)	127 (32.9)	.49	142 (58.2)	<.001	118 (69.4)	<.001
<i>ADAM12</i> (rs3740199)							
Genotype							
GG	83 (41.5)	75 (38.9)	Reference	29 (23.8)	Reference	20 (23.5)	Reference
GC	92 (46.0)	85 (44.0)	.99	51 (41.8)	.13	44 (51.8)	.04
CC	25 (12.5)	33 (17.1)	.28	42 (34.4)	<.001	21 (24.7)	.002
Allele							
G	258 (64.5)	235 (60.9)	Reference	109 (44.7)	Reference	84 (49.4)	Reference
C	142 (35.5)	151 (39.1)	.33	135 (55.3)	<.001	86 (50.6)	.001
<i>ADAM12</i> (rs1871054)							
Genotype							
GG	56 (28.0)	44 (22.9)	Reference	40 (32.8)	Reference	24 (28.2)	Reference
GC	96 (48.0)	110 (56.9)	.16	59 (48.4)	.66	45 (52.9)	.89
CC	48 (24.0)	39 (20.2)	.97	23 (18.8)	.29	16 (18.9)	.63
Allele							
G	208 (52.0)	198 (51.3)	Reference	139 (56.9)	Reference	93 (54.7)	Reference
C	192 (48.0)	188 (48.7)	.90	105 (43.1)	.25	77 (45.3)	.62

^aCompared with the control group.

Table 5

Effect of *ADAM12* single-nucleotide polymorphisms on *ADAM12* messenger RNA expression in the sputum of the asthmatic group

Gene	GG	GC	CC	P value
<i>ADAM12</i> (rs3740199)				.02
No.	15	28	11	
Mean (SD)	0.325 (0.23)	0.476 (0.34)	0.651 (0.13)	
<i>ADAM12</i> (rs1871054)				.03
No.	18	26	10	
Mean (SD)	0.331 (0.21)	0.482 (0.41)	0.663 (0.09)	

were significantly increased in the sputum from asthmatic patients compared with controls ($P < .001$). Of interest, *ADAM33* mRNAs were not expressed in the asthmatic or control groups.

We studied the effect of *ADAM12* SNPs on *ADAM12* expression. Surprisingly, we found that the mean values of the *ADAM12* relative expression were significantly higher for homozygous CC (variant type) compared with homozygous GG (wild type) for *ADAM12* (rs3740199 and rs1871054) in the asthmatic group ($P \leq .001$ for each) (Table 5).

Discussion

The present study was conducted to analyze the association of specific *ADAM33* gene polymorphisms F+1 (rs5118980) and ST+4 (rs44707) and *ADAM12* gene polymorphisms (rs3740199 and rs1871054) with asthma risk and severity in Egyptian children. This is the first study, to our knowledge, to analyze the association of *ADAM12* gene polymorphisms with asthma. We evaluated the association between *ADAM12* with asthma risk and severity in Egyptian children. Two SNPs of the *ADAM12* gene were investigated. The *ADAM12* rs3740199 (G > C) is located in the second exon, whereas the *ADAM12* rs1871054 (C > T) is located in the 11th intron. In the present study, we found that the homozygous mutant genotype (CC) and the mutant allele C of *ADAM12* (rs3740199) were significantly associated with asthma risk and severity in moderate and severe subgroups.

Kernay et al²⁴ and Valdes et al²⁶ found that *ADAM12* (rs3740199) gene polymorphisms may help in the diagnosis and progression of knee osteoarthritis, identifying the role of *ADAM12* (rs3740199) in inflammatory diseases. On the other hand, we failed to detect an association between rs1871054 and asthma risk or severity. This finding differs from the results of the study by Valdes et al,²⁶ who found that *ADAM12* rs1871054 plays an important role in the development of knee osteoarthritis. In the present study, we found that *ADAM12* expression was significantly higher in homozygous CC (variant type) compared with homozygous GG (wild type) of *ADAM12* (rs3740199 and rs1871054) in the cells obtained from the sputum of asthmatic patients. Our results indicated that *ADAM12* polymorphisms could play a role in the upregulation of *ADAM12* expression.

In agreement with our study, Paulissen et al²⁵ observed an increased expression of *ADAM12* mRNA in induced sputum from patients with asthma compared with controls. Estrella et al²⁷ reported that *ADAM12* expression was strongly increased in airway epithelial cells from patients with allergic rhinitis after allergen challenge. The secreted form of *ADAM12* provokes myoblast fusion in vivo by binding to the muscle-specific actin-binding protein, α -actinin 2.²⁸ It could be implicated in converting airways smooth muscle cells from acting as multiple units to a single contractile unit,²⁹ a property relevant to the pathogenesis of bronchial hyperresponsiveness in asthma.

Concerning *ADAM33* SNPs analysis, we found that the F+1 homozygous mutant genotype (AA), ST+4 heterozygous and homozygous mutant genotype (AC and CC), and mutant alleles of

both polymorphisms were significantly associated with asthma risk and with severity in moderate and severe subgroups. The ST+4 SNP lies in the intron separating the transmembrane and cytoplasmic domains, whereas the F+1 lies in the catalytic domain of the *ADAM33* gene. Some of the disease-related *ADAM33* SNPs encode amino acid changes.³⁰ Other SNPs are located in the noncoding regions and may affect proliferation of myofibroblasts and smooth muscle and/or inflammation of the airways by alternative splicing and splicing efficiency of mRNA turnover.³⁰ *ADAM33* polymorphisms may accelerate the proliferation of smooth muscle cells and fibroblasts, leading to bronchial hyperactivity and subepithelial fibrosis.³¹

Similar to our results, Wernar et al¹⁶ observed a statistically significant association of asthma with SNPs F+1, ST+4, and ST+5 (not involved in our study) in a German family-based study. Another case-control study found a significant association of F+1, S2, ST+4, ST+5, and V4 with asthma in Indian children.¹⁹ Howard et al³² assessed the association of ST+4 and V4 with asthma and found significant associations of SNPs in the studied population, but no single SNP was associated with a specific asthma phenotype. Other studies found a positive association between *ADAM33* ST+4 SNPs with asthma susceptibility in Thai and Japanese populations.^{17,18} A meta-analysis study confirmed the positive association between the *ADAM33* ST+4 polymorphism with asthma susceptibility in Asians.³³ The F+1 polymorphism has a positive association with asthma in a UK population, a German family-based study, and a Dutch birth cohort.^{16,34} Furthermore, Jie et al³⁵ found that F+1 was associated with asthma susceptibility in the East China Han population, and the genetic association is stronger in patients with high-severity asthma. A meta-analysis examined the association of *ADAM33* gene polymorphisms with the risk of asthma, and the results indicated that ST+4 was not associated with asthma, whereas F+1 was a risk factor for asthma, especially in the Asian population.³⁶ However, in contrast to our study, many studies found no association between *ADAM33* SNPs and childhood asthma.^{37–40} Recently, Klaassen et al⁴¹ found that the minor allele of *ADAM33* F+1 (rs511898) was not associated with childhood asthma. The controversy between our study and others may be due to the differences in sample sizes, heterogeneity of populations, and different environmental exposures.

In the current study, we were not able to detect expression of *ADAM33* in the sputum of asthmatic patients. This finding may be attributed to the fact that *ADAM33* is expressed in the lung fibroblasts and the bronchial smooth muscle cells but not in the bronchial epithelial cells and the inflammatory cells.^{10,42} On the contrary, Lee et al⁴³ found that the amounts of *ADAM33* protein in bronchoalveolar lavage from patients with asthma correlate with lung function loss. Tripathi et al⁴⁴ detected a higher expression of *ADAM33* protein in the bronchial epithelium of asthmatic patients compared with controls. The mechanisms for airway remodeling of *ADAM33* are not clear, but it has been suggested that *ADAM33* may affect the epithelial-mesenchymal trophic unit. Lin et al⁴⁵ suggested that *ADAM33* is a mediator of airway smooth muscle cell dysfunction in asthma.

The major limitation of our study was a lack of replication. However, the present study fulfills most of the criteria of a good genetic association study.⁴⁶ All the study groups were in Hardy-Weinberg equilibria. The sample size was predetermined for *ADAM* polymorphisms to avoid type I error.

In conclusion, our results suggested that *ADAM33* and *ADAM12* gene polymorphisms may modify individual susceptibility to develop childhood asthma in Egyptian children. There were significant associations between *ADAM33* F+1, ST+4, and *ADAM12* rs3740199 and disease severity. Lastly, *ADAM12* polymorphisms may affect *ADAM12* expression in asthmatic children.

References

- [1] Maziak W, Behrens T, Brasky TM, et al. Are asthma and allergies in children and adolescents increasing? results from ISAAC phase I and phase III surveys in Munster, Germany. *Allergy*. 2003;58:572–579.
- [2] Su MW, Tsai CH, Tung KY, et al. GSTP1 is a hub gene for gene-air pollution interactions on childhood asthma. *Allergy*. 2013;68:1614–1617.
- [3] Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. *J Allergy Clin Immunol*. 2011;128:451–462.
- [4] Holgate ST, Davies DE, Powell RM, Howarth PH, Haitchi HM, Holloway JW. Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur Respir J*. 2007;29:793–803.
- [5] Ortiz RA, Barnes KC. Genetics of allergic diseases. *Immunol Allergy Clin North Am*. 2015;35:19–44.
- [6] Huovila AP, Turner AJ, Peltto-Huikko M, Karkkainen I, Ortiz RM. Shedding light on ADAM metalloproteinases. *Trends Biochem Sci*. 2005;30:413–422.
- [7] Yamamoto S, Higuchi Y, Yoshiyama K, et al. ADAM family proteins in the immune system. *Immunol Today*. 1999;20:278–284.
- [8] Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F, Matsushima K. Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. *J Biol Chem*. 1997;272:556–562.
- [9] Ito I, Laporte JD, Fiset PO, et al. Downregulation of a disintegrin and metalloproteinase 33 by IFN-gamma in human airway smooth muscle cells. *J Allergy Clin Immunol*. 2007;119:89–97.
- [10] Umland SP, Garlisi CG, Shah H, et al. Human ADAM33 mRNA expression profile and post-transcriptional regulation. *Am J Respir Cell Mol Biol*. 2003;29:571–582.
- [11] Garlisi CG, Zou J, Devito KE, et al. Protein maturation and localization. *Biochem Biophys Res Commun*. 2003;301:35–43.
- [12] Hirota T, Hasegawa K, Obara K, et al. Association between ADAM33 polymorphisms and adult asthma in the Japanese population. *Clin Exp Allergy*. 2006;36:884–891.
- [13] Gosset P, Tillie-Leblond I, Malaquin F, Durieu J, Wallaert B, Tonnel AB. Interleukin-8 secretion in patients with allergic rhinitis after an allergen challenge: interleukin-8 is not the main chemotactic factor present in nasal lavages. *Clin Exp Allergy*. 1997;27:379–388.
- [14] Brzoska E, Bello V, Darribere T, Moraczewski J. Integrin alpha3 subunit participates in myoblast adhesion and fusion in vitro. *Differentiation*. 2006;74:105–118.
- [15] Rocks N, Estrella C, Paulissen G, et al. The metalloproteinase ADAM-12 regulates bronchial epithelial cell proliferation and apoptosis. *Cell Prolif*. 2008;41:988–1001.
- [16] Werner M, Herbon N, Gohlke H, et al. Asthma is associated with single-nucleotide polymorphisms in ADAM 33. *Clin Exp Allergy*. 2004;34:26–36.
- [17] Noguchi E, Ohtsuki Y, Tokunaga K, et al. ADAM33 polymorphisms are associated with asthma susceptibility in a Japanese population. *Clin Exp Allergy*. 2006;36:602–608.
- [18] Thongnarm T, Jameekornrak A, Limwongse C, et al. Association between ADAM33 polymorphisms and asthma in a Thai population. *Asian Pac J Allergy Immunol*. 2008;26:205–211.
- [19] Awasthi S, Tripathi P, Ganesh S, Husain N. Association of ADAM33 gene polymorphisms with asthma in Indian children. *J Hum Genet*. 2011;56:188–195.
- [20] Global Initiative for Asthma. Global strategy for asthma management and prevention. 2011. <http://www.ginasthma.org/>. Accessed May 10, 2013.
- [21] Zar HJ, Tannenbaum E, Hanslo D, Hussey G. Sputum induction as a diagnostic tool for community-acquired pneumonia in infants and young children from a high HIV prevalence area. *Pediatr Pulmonol*. 2003;36:58–62.
- [22] Cataldo D, Munaut C, Noël A, et al. MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol*. 2000;123:259–267.
- [23] Tripathi P, Awasthi S, Prasad R, Husain N, Ganesh S. Association of ADAM33 gene polymorphisms with adult-onset asthma and its severity in an Indian adult population. *Genetics*. 2011;90:2.
- [24] Kerna I, Kisand K, Tamm AE, Lintrop M, Veske K, Tamm AO. Missense single nucleotide polymorphism of the ADAM12 gene is associated with radiographic knee osteoarthritis in middle-aged Estonian cohort. *Osteoarthritis Cartilage*. 2009;17:1093–1098.
- [25] Paulissen G, Rocks N, Quesada-Calvo F, et al. Expression of ADAMs and their inhibitors in sputum from patients with asthma. *Mol Med*. 2006;12:171–179.
- [26] Valdes AM, Hart DJ, Jones KA, et al. Association study of candidate genes for the prevalence and progression of knee osteoarthritis. *Arthritis Rheum*. 2004;50:2497–2507.
- [27] Estrella C, Rocks N, Paulissen G, et al. Role of A disintegrin and metalloprotease-12 in neutrophil recruitment induced by airway epithelium. *Am J Respir Cell Mol Biol*. 2009;41:449–458.
- [28] Gilpin BJ, Loebel F, Mattei MG, et al. A novel, secreted form of human ADAM 12 (meltrin alpha) provokes myogenesis in vivo. *J Biol Chem*. 1998;273:157–166.
- [29] Hirst SJ. Airway smooth muscle cell culture: application to studies of airway wall remodelling and phenotype plasticity in asthma. *Eur Respir J*. 1996;9:808–820.
- [30] Haitchi HM, Powell RM, Shaw TJ, et al. ADAM33 expression in asthmatic airways and human embryonic lungs. *Am J Respir Crit Care Med*. 2005;171:958–965.
- [31] Shapiro SD, Owen CA. ADAM-33 surfaces as an asthma gene. *N Engl J Med*. 2002;347:936–938.
- [32] Howard TD, Postma DS, Jongepier H, et al. Association of disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. *J Allergy Clin Immunol*. 2003;112:717–722.
- [33] Song GG, Kim JH, Lee YH. Association between ADAM33 S2 and ST+4 polymorphisms and susceptibility to asthma: a meta-analysis. *Gene*. 2013;15:72–78.
- [34] Reijmerink NE, Kerkhof M, Koppelman GH, et al. Smoke exposure interacts with ADAM33 polymorphisms in the development of lung function and hyperresponsiveness. *Allergy*. 2009;64:898–904.
- [35] Jie Z, Hu Z, Bai C, et al. ADAM33 gene polymorphisms associate with asthma susceptibility and severity in East China Han population. *J Asthma*. 2011;48:979–985.
- [36] Liang S, Wei X, Gong C, Wei J, Chen Z, Deng J. A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. *Hum Immunol*. 2013;74:648–657.
- [37] Lind DL, Choudhry S, Ung N, et al. ADAM33 is not associated with asthma in Puerto Rican or Mexican populations. *Am J Respir Crit Care Med*. 2003;168:1312–1316.
- [38] Raby BA, Silverman EK, Kwiatkowski DJ, Lange C, Lazarus R, Weiss ST. ADAM33 polymorphisms and phenotype associations in childhood asthma. *J Allergy Clin Immunol*. 2004;113:1071–1078.
- [39] Kedda MA, Duffy DL, Bradley B, O'Hehir RE, Thompson PJ. ADAM33 haplotypes are associated with asthma in a large Australian population. *Eur J Hum Genet*. 2006;14:1027–1036.
- [40] Schedel M, Depner M, Schoen C, et al. 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.
- [41] Klaassen EM, Penders J, Jöbsis Q, et al. An ADAM33 polymorphism associates with progression of preschool wheeze into childhood asthma: a prospective case-control study with replication in a birth cohort study. *PLoS One*. 2015;10(3):e0119349.
- [42] Van Eerdewegh P, Little RD, Dupuis J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature*. 2002;418:426–430.
- [43] Lee JY, Park SW, Chang HK, et al. A disintegrin and metalloproteinase 33 protein in patients with asthma: relevance to airflow limitation. *Am J Respir Crit Care Med*. 2006;173:729–735.
- [44] Tripathi P, Awasthi S, Husain N, et al. Increased expression of ADAM33 protein in asthmatic patients as compared to non-asthmatic controls. *Indian J Med Res*. 2013;137:507–514.
- [45] Lin F, Song A, Wu J, et al. ADAM33 protein expression and the mechanics of airway smooth muscle cells are highly correlated in ovalbumin-sensitized rats. *Mol Med Rep*. 2013;8:1209–1215.
- [46] Hattersley AT, McCarthy MI. What makes a good genetic association study? *Lancet*. 2005;366:1315–1323.