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High frequency of a deletion polymorphism of the angiotensin-converting enzyme gene in asthma

Joelle Benessiano^a [[MEDLINE LOOKUP](#)]

Bruno Crestani^{b,c} [[MEDLINE LOOKUP](#)]

Fouzi Mestari^a [[MEDLINE LOOKUP](#)]

Wassila Klouche^a [[MEDLINE LOOKUP](#)]

Françoise Neukirch^c [[MEDLINE LOOKUP](#)]

Salima Hacein-Bey^a [[MEDLINE LOOKUP](#)]

Genevieve Durand^a [[MEDLINE LOOKUP](#)]

Michel Aubier^{b,c} [[MEDLINE LOOKUP](#)]

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Background: An insertion-deletion polymorphism of the angiotensin-converting enzyme (ACE) gene has been shown to be associated with levels of ACE. Because ACE is heavily expressed in the lungs and plays a key role in the metabolism of angiotensin II and bradykinin, which are potentially involved in the pathogenesis of asthma, we tested the hypothesis of an association between the polymorphism of the ACE gene and asthma.

Methods: Seventy-nine patients with asthma, 54 healthy subjects, and 33 patients with nonasthmatic lung disease were studied. Pulmonary function tests were performed in patients with asthma, and the ACE genotypes were determined in all subjects by the polymerase chain reaction.

Results: The ACE genotype distribution was similar in healthy subjects and in patients without asthma. By contrast, the population of patients with asthma was characterized by a higher prevalence of the DD genotype of ACE (odds ratio, 2.09; 95% confidence interval, 1.05 to 4.16; $p = 0.023$). No difference in pulmonary function test results was detected in asthmatic patients according to the distribution of ACE genotypes.

Conclusion: This study reports an association between the DD genotype of ACE and asthma, which is not related to the degree of airway obstruction. These results need to be confirmed by a larger case-control study. (J Allergy Clin Immunol 1997;99:53-7.)

AI Angiotensin II

ACE Angiotensin-converting enzyme

CI Confidence interval

D Deletion

FVC Forced vital capacity

I Insertion

PCR Polymerase chain reaction

(Click on a term to search this journal for other articles containing that term.)

Key Words: [Genetics](#), [bradykinin](#), [substance P](#), [tachykinin](#)

Asthma is a human pulmonary disease characterized by chronic inflammation of the airways and bronchial hyperresponsiveness.^{1 2} The findings of previous epidemiologic and family studies suggest that asthma is a multifactorial disease influenced by both genetic and environmental factors.^{3 4} Although the nature of the genetic factors remains unknown, it has become evident that the airway narrowing in asthma is a consequence of a special type of chronic inflammation of the airway wall. Among the mediators implicated in the genesis of this inflammatory process, the role of some naturally occurring inflammatory peptides, such as bradykinin and related peptides has been described extensively.^{5 6} Bradykinin and substance P, which have profound inflammatory effects, are inactivated at least in part, by angiotensin I-converting enzyme (ACE) (dipeptylcarboxy-peptidase

I, peptidyl-peptidase A, kininase II; EC 3-4-15-1).^{7 8} ACE has been identified as a membrane-bound enzyme in several types of cells and is also secreted as a so-called soluble form in plasma. In addition to the inactivation of bradykinin and tachykinin peptides, the other major function of ACE is the conversion of angiotensin I into angiotensin II (A II),^{7 9} a compound that may also be involved in the pathogenesis of asthma by interacting with airway smooth muscle.¹⁰

An insertion/deletion polymorphism of the ACE gene has been shown to be associated with differences in plasma ACE level¹¹ and with the level of expression of membrane-bound ACE in human peripheral mononuclear cells.¹² This suggests that the polymorphism of the ACE gene could affect ACE expression in other types of cells and could participate in the control of ACE regulation.

Because ACE is heavily expressed in the lungs¹³ and plays a key role in the metabolism of peptides possibly involved in the pathogenesis of asthma, we asked whether the polymorphism of the ACE gene could be implicated as one of the genetic factors in patients with asthma. Thus the aim of this study was to test the hypothesis of an association between the insertion/deletion polymorphism of the ACE gene and asthma in an adult population.

Methods

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Study population

Seventy-nine consecutive patients with asthma (30 men and 49 women), aged 44 ± 19 years (mean $\pm$ SD), were studied. Patients were evaluated in the outpatient clinic for chronic asthma or in the hospitalization unit for acute exacerbation of asthma. Asthma was diagnosed on clinical grounds as the episodic occurrence of coughing, dyspnea, and wheezing according to the American Thoracic Society official statement.¹⁴

Fifty-four healthy subjects (19 men and 35 women), aged 42 ± 10 years (mean $\pm$ SD), who were free of lung disease and had no clinical evidence of personal or familial atopy or asthma were recruited from the medical and laboratory staff and served as control subjects.

Thirty-three consecutive patients (25 men and 8 women), aged 53 ± 15 years (mean $\pm$ SD), who were admitted to the pulmonary unit or evaluated in the outpatient clinic for nonasthmatic lung disease and who had no clinical evidence of personal or familial atopy or asthma were also studied. The distribution of lung disease was as follows: lung cancer ($n = 14$), emphysema ($n = 5$), bronchiectasis ($n = 3$), bacterial pneumonia ($n = 3$), tuberculosis ($n = 2$), cryptogenic organizing pneumonia ($n = 1$), pneumothorax ($n = 1$), venoocclusive lung disease ($n = 1$), Langerhans cell granulomatosis ($n = 1$), sarcoidosis ($n = 1$), and pleural effusion ($n = 1$).

Patients and control subjects with clinical evidence of cardiovascular disease were excluded because of the possible association between the insertion/deletion polymorphism of the ACE gene and the development of ischemic heart disease.^{15 17} All patients and healthy subjects were Caucasians and gave informed consent to participate in the study.

Pulmonary function tests

In patients with asthma, a flow volume curve was performed to determine the degree of airway obstruction at least 1 month after an acute exacerbation of asthma. The FEV₁ and the forced vital capacity (FVC) were measured, and the FEV₁/FVC ratio was calculated.

Determination of ACE genotypes

The ACE gene contains a polymorphism based on the presence (or insertion [I]) or absence (or deletion [D]), in intron 16, of a 287 bp nonsense DNA domain, which results in three different genotypes: DD and II homozygotes and ID heterozygotes.¹⁸

Genomic DNA was prepared from frozen peripheral blood by using standard procedures,¹⁹ and the D and I alleles were identified, as previously described, on the basis of polymerase-chain-reaction (PCR) amplification of the respective fragments of the ACE gene.²⁰ PCR products were separated by agarose gel electrophoresis, and DNA was visualized by ethidium bromide staining. Subjects were then classified, according to the absence or presence of the 287 bp insertion in intron 16 of the ACE gene, as DD or II homozygotes or as ID heterozygotes.

Statistical analysis

The frequencies of the alleles and genotypes among all healthy subjects and all patients were counted. The frequencies of the genotype DD in each group were compared by means of the chi square test. Odds ratios with 95% confidence intervals (CIs) were calculated as a measure of the association of the ACE genotype with the phenotype of asthma; *p* values less than 0.05 are presented and considered significant.

Data are expressed as mean $\pm$ SD. Differences between the studied groups defined according to their genotype of ACE (as DD or non-DD) were evaluated with the unpaired *t* test with 95% CI.

Results

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Frequencies of alleles and genotypes

Among the healthy population, the frequencies of the I and D alleles were 0.44 and 0.56, respectively; and the distribution of the DD, II, and ID genotypes was 27%, 15%, and 58%, respectively (Table I).

Table I. Frequencies of the genotypes and alleles of the insertion/deletion polymorphism of the ACE gene

Frequency (%)

	Healthy subjects (n = 54)	Patients without asthma (n = 33)	All subjects without asthma (n = 87)	Patients with asthma (n = 79)
ACE genotypes and alleles (%)				
DD	27	27	27	47
ID	58	61	59	38
II	15	12	14	15
D	56	57	57	66
I	44	43	43	34

Comparison of patients without asthma versus healthy subjects: chi square = 0.04; $p = 0.96$; odds ratio = 2.06; 95% CI = 0.83 to 5.16
Comparison of patients with asthma versus all subjects without asthma: chi square = 5.14; $p = 0.023$; Odds ratio = 2.09; 95% CI = 1.05 to 4.16

Among the population of patients with lung disease without clinical evidence of asthma, the frequencies of the I and D alleles were 0.43 and 0.57, respectively; and the distribution of the DD, II, and ID genotypes was 27%, 12%, and 61%, respectively. The frequencies of the I and D alleles and the DD, II, and ID genotype distribution were not significantly different between healthy subjects and patients without asthma (Table I [15 21](#)) and were in agreement with data previously reported for other Caucasian control populations. ^{15 21} Therefore we pooled the data from our two populations of patients without asthma and obtained a unique control population of nonasthmatic subjects ($n = 87$). Among this control population, the observed frequencies of the I and D alleles were 0.43 and 0.57, respectively; and the distribution of the DD, II, and ID genotypes was 27%, 14%, and 59%, respectively.

Among the population of patients with asthma, the frequencies of alleles I and D were 0.34 and 0.66, respectively; and the distribution of the DD, II, and ID genotypes was 47%, 15%, and 38%, respectively. The asthmatic population was characterized by a higher prevalence of the DD genotype (chi square = 5.14; $p = 0.023$; odds ratio 2.09 [95% CI = 1.05-4.16]).

Pulmonary function tests

The FEV₁ and FEV₁/FVC ratio were not statistically different in the patients with asthma when they were classified into two groups according to their genotype (DD genotype and II and ID genotypes [defined as non-DD]) (Table II).

Table II. Pulmonary function tests measured in 79 patients with asthma who were classified according to their ACE genotype (DD or non-DD)

ACE genotype	FEV ₁ (% of predicted)	FEV ₁ /FVC (%)
DD	79.5 ± 23.6	68.7 ± 13.5
non-DD	76.5 ± 22.8	65.9 ± 15.8
<i>p</i> Value	0.62 (NS)	0.46 (NS)

NS, Not significant.

Discussion

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This study is the first, to our knowledge, to report evidence for an association between the insertion/deletion polymorphism of the ACE gene and asthma. The frequency of the DD genotype of ACE was higher in a population of patients with asthma than in a nonasthmatic control population. The odds ratio, an estimate of the relative risk of developing asthma, between patients with the DD genotype and those with the II or ID genotypes was 2.09, suggesting that the DD genotype of ACE may be a genetic risk factor for developing asthma.

A possible problem in such a study is the misclassification of the populations. Asthma was diagnosed by the episodic occurrence of coughing, dyspnea, and wheezing, according to the guidelines of the American Thoracic Society.¹⁴ Therefore a misclassification of the studied populations may arise from the use of such a clinical definition of asthma. First, some patients with poorly defined symptoms of asthma could be classified as non-asthmatic. However, if this was the case, it was not quantitatively important because we took care to exclude, from our nonasthmatic population, all patients with a personal or familial history of atopy or asthma. Second, although the diagnosis of asthma was made by one or more experienced pulmonologists, an overdiagnosis of asthma was possible in patients without asthma. This event is also unlikely because a large majority of the patients had chronic asthma and were followed for up several months in our unit. Those who were seen for the first time arrived in the emergency department of our hospital with a typical attack of asthma and had a history of similar episodes. Third, because all participating patients with asthma were exclusively recruited from the hospital, they could not be representative of the global population of patients with asthma. Indeed, it could be possible that the DD genotype of ACE is associated with a higher degree of severity of asthma. However, our results do not support this hypothesis because the obstructive ventilatory defect, defined as the FEV₁/FVC ratio, was not different in the patients with asthma regardless of whether they carried the DD genotype. Finally, we observed that the distribution of genotypes was not different in men and women, both in control subjects and patients (data not shown). Also, all subjects participating in this study were Caucasians and lived in France. Therefore it is unlikely that population heterogeneity could explain our results. It is worth noting that the allele frequencies of the ACE gene in our control population were similar to previously reported data obtained in other Caucasian control populations.^{15 21} Thus a sample bias in the choice of the control population seems to be unlikely in this study. However, although our control group was similar to the group of patients with asthma in terms of age, sex ratio, and ethnic characteristics (all were Caucasians), the subjects did not constitute a random control group. Furthermore, the patient sample tested in this study was rather small, and the level of significance was borderline. Our results must be confirmed by a larger case-control study to confirm

that ACE represents a candidate gene in asthma and to establish whether our results can be applied to other ethnic or national groups.

Another problem is a possible association of the ACE genotype with a confounding variable. Indeed, the ACE I/D polymorphism could be a genetic marker of an unknown functional variant located within or near the ACE gene. Further studies will be necessary to determine the involvement of ACE polymorphism in a scenario of multigene defects in asthma.

Although our study shows a positive association between differences in the DNA sequence of the ACE gene and asthma, it does not identify the mechanism by which the DD genotype may affect asthmatic patients. However, we can hypothesize that the high plasma and cell levels of ACE that are strongly associated with the DD genotype^{11 12}, could participate in the pathogenesis of asthma, particularly through the induction of AII. Indeed, it has been shown previously that AII may contribute to bronchoconstriction during acute severe asthma,¹⁰ suggesting either a direct effect on airway smooth muscle or an indirect effect through the release of other mediators of bronchoconstriction such as endothelin.²² AII is also known to promote the growth of cultured smooth muscle cells in vitro.²³ In view of these observations, it can be proposed that a chronic increased expression of ACE in patients carrying the DD genotype could affect bronchial reactivity and favor the thickening of the walls of the airways by contributing to airway smooth muscle hypertrophy.

Conversely, the high levels of bradykinin in plasma and bronchoalveolar lavage fluid reported in patients with asthma^{24 25}, seem to be in apparent contradiction with the high frequency of the DD genotype reported in the patients with asthma in this study. However, it can be postulated that the increased level of ACE activity in patients with asthma carrying the DD genotype could be quantitatively insufficient and/or qualitatively inefficient to counterbalance the oversynthesis of bradykinin occurring in patients with asthma. Clearly, the physiopathologic mechanisms by which the I/D polymorphism of the ACE gene may be involved in the pathogenesis of asthma remains to be determined in further studies.

In summary, this study reports the first evidence for an association between the I/D polymorphism of the ACE gene and asthma. The main finding is a higher frequency of the DD genotype in patients with asthma than in subjects without asthma, suggesting that DNA sequence differences in the ACE gene may be involved in the genesis of asthma. Moreover, the DD genotype of ACE in patients with asthma does not appear to be related to the degree of severity of airway obstruction. However, our results must be confirmed in a larger case-control study to determine the involvement of the deletion polymorphism of the ACE in the pathogenesis of asthma.

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Lydie Hollá, MUDr, Anna Věšková, MUDr, CSc, Vladimír Znojil, RNDr, CSc, Lenka Šišková, MUDr, Jiří Vácha, MUDr, DrSc

ABSTRACT

FULL TEXT