

Association of 3 gene polymorphisms with atopic diseases

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Background: Various peptidases, including angiotensin-converting enzyme (ACE), inactivate some inflammatory peptides that are considered to influence the pathogenesis of atopic diseases. This enzyme is also involved in the conversion or activation of 2 bronchoconstriction mediators: angiotensin II from angiotensinogen and endothelin (ET), respectively.

Objective: We tested a hypothesis that asthma or other atopic diseases are associated with insertion/deletion ACE, M235T angiotensinogen, and TaqI ET-1 gene polymorphisms.

Methods: A case-control approach was used in the study.

Healthy subjects (141 persons) were used as control subjects, and 231 patients with histories of atopic asthma, allergic rhinitis, atopic dermatitis, or a combination thereof were studied.

ACE genotype was determined by PCR, angiotensinogen M235T and ET-1 by PCR, and restriction analysis by AspI and TaqI, respectively.

Results: We found the significant association of the insertion/deletion polymorphism of the ACE, as well as that of M235T polymorphism of the angiotensinogen genes, with the group of patients with atopic diseases ($P = .0025$ and $P = .0204$, respectively). No difference was proved for the intron 4 (position 8000) polymorphism in the ET-1 gene when comparing the atopic patients with the control group ($P = .1774$). A significant difference was found between groups of patients with both asthma and rhinitis and patients without both respiratory atopic diseases ($P = .0033$).

Conclusion: It follows that the examined polymorphisms in the genes for ACE, angiotensinogen, and ET-1 could participate in the etiopathogenesis of atopic diseases. (*J Allergy Clin Immunol* 1999;103:702-8.)

Key words: Angiotensin-converting enzyme, angiotensinogen, endothelin-1, genes, polymorphism, enzymes, asthma bronchiale, atopy

Atopy is a genetic predisposition toward the development of immediate hypersensitivity reactions against common environmental antigens. Atopy commonly

Abbreviations used

ACE: Angiotensin-converting enzyme
D: Deletion
ET: Endothelin
I: Insertion

exists without overt clinical manifestation, but it is also associated with a heterogeneous group of clinical disorders, including asthma bronchiale, allergic rhinitis, and atopic dermatitis.

Allergy and asthma, like coronary heart disease, hypertension, and diabetes, are common diseases. Although asthma and atopy both have a strong genetic component, they do not display a classic Mendelian mode of inheritance attributable to a single gene locus.¹ They are complex genetic disorders in which multiple interactions from numerous genes, combined with environmental influences, are involved. Allergic diseases are clearly defined in accordance with clinical manifestation, and their definition according to laboratory results is difficult. These laboratory results are variable in time, and sometimes they lack a tight relation to relevance of disease. Many factors, such as lack of penetrance and variable expressivity, environmental effects, high gene frequency, and genetic heterogeneity, may complicate the study of the genetics of atopy.² Although discovering the genetics underlying asthma and allergy is difficult, it appears that they are closely interrelated, with most asthmatic patients having clinical and serologic evidence of atopy.

On the assumption that in all symptoms, including atopy, the same genes are involved, at least particularly on the basis of linkage studies, the qualified estimate can be made that only a limited number of genes seriously influences the pathophysiology of the diseases mentioned above. Because the incidence of these diseases in populations is high, the alleles of polymorphisms of these genes would be frequent. That is why a limited (and statistically predictable) number of probands might be sufficient for the detection of the influence of the polymorphisms.

Asthma bronchiale is a pulmonary disease, which is characterized by chronic inflammation.^{3,4} This chronic inflammation is responsible for increased airway hyperresponsiveness to a variety of stimuli and for the recurrent symptoms and airflow limitation characteristics of asthma.⁵ Chronic inflammation also triggers pathophysi-

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ologic changes occurring in the upper airways of patients with allergic rhinitis and in the skin of those with atopic dermatitis.

Some naturally occurring inflammatory peptides, such as bradykinin, substance P, and others, are involved in the secondary inflammatory response.⁶ These peptides are partially inactivated by angiotensin-converting enzyme (ACE) (peptidyl-peptidase A, dipeptylcarboxy-peptidase I, kininase II, EC 3-4-15-1). The levels of tissue⁷ and circulating ACE activity are under tight genetic control.⁸ An association between the ACE polymorphism and ACE levels was found. The highest level was observed in homozygotes for the deletion (D) allele, and the lowest level was observed in persons with the insertion (I) allele. An intermediate level was found in heterozygotes.⁹⁻¹¹ I/D ACE polymorphism not only modulates the level of plasma ACE but was also shown to modulate the intracellular level of ACE in T lymphocytes.¹² Apart from inactivating the above-mentioned peptides, ACE is also involved in the conversion of angiotensin I to angiotensin II. Augmented secretion of proinflammatory cytokines (eg, IL-1) in the site of inflammation results in an enhanced angiotensinogen expression.¹³⁻¹⁵ Expression of angiotensinogen by leukocytes may provide a mobile angiotensin-generating system of potential importance in the regulation of local inflammatory responses. It is possible that local generation of angiotensin II by infiltrating leukocytes^{16,17} in a tissue undergoing an inflammatory response might, by means of the intrinsic renin-angiotensin system, modulate activation tissue blood flow, increase vascular permeability and fluid filtration across capillaries, and increase granulomatous responses.^{18,19} Angiotensin II is not only a vasoactive peptide, but it may also increase vascular smooth muscle growth.^{20,21} Angiotensin II, as well as IL-1, thrombin, bradykinin, or hypoxia, can stimulate endothelin-1 (ET-1) release.²² ET was originally identified as a potent 21-residue vasoconstrictor peptide in vascular endothelial cells, and it is presently known to be produced by many different tissues.²³⁻²⁶ ET-1 may be involved in the pathogenesis of asthma by causing bronchial smooth muscle constriction and airway remodeling and activating alveolar macrophages. The inflammatory process may induce ET-1 production, thus setting off a cytokine cascade, which may in turn promote cellular infiltration and intensify the chronic phase of airway inflammation. Bronchial epithelial cells represent an important source of ET-1 in this state, and an increased release of epithelial cell-derived ET-1 may contribute to the genesis of subepithelial fibrosis by promoting fibroblast proliferation and collagen production.²⁷ Although immunoreactive ET-1 and mRNA for ET-1 were detected from cells of epithelial origin,²⁸⁻³⁰ it has not been characterized as an autocrine growth factor for epithelial cells. On the other hand, normal human keratinocytes were found to be stimulated to human keratinocyte growth and DNA synthesis, and their differentiation was suppressed by ET-1.

Although genes coding ACE and angiotensinogen, in contrast to ET-1, have not been supposed to be candidate genes for atopy, there are several pathophysiologic rea-

sons for testing a hypothesis that asthma or other atopic diseases may be associated with I/D ACE (as a modulator of proliferation of hemopoietic cells and a neuromodulator), M235T angiotensinogen (as an intersection of the stress activation axis and both IL-1 and acute-phase protein activation), and T/C ET-1 (both as a vasoconstrictive and proliferative factor) gene polymorphisms.

METHODS

Study population

Probands. White subjects of Czech nationality ($n = 372$) were included in the cross-sectional comparative study. Probanders were selected by using a detailed questionnaire modified from the American Thoracic Society respiratory questionnaire³¹ regarding lifetime symptoms suggestive of asthma, rhinitis, and eczema, with additional questions on symptoms and therapy, as well as on other types of disease, especially cardiovascular disease. The questionnaires were filed by the physician by means of anamnesis and data available in the documentation. Phenotype status was assigned without previous knowledge of genotypes by 2 independent investigators.

The control group consisted of 141 healthy subjects (74 men and 67 women) aged 41.3 ± 15.7 years (mean $\pm$ SD) without clinical symptoms of any lung disease and/or immunologic symptoms. The subjects had no evidence of personal history of atopy, cardiac disorders, or hypertension, and they were taking no medications. The subjects were recruited from those persons registered in general practitioners' practices. In the control sample laboratory tests and skin prick tests were unobtainable. Hypertension was excluded by means of repeated blood pressure measurement (according to World Health Organization criteria).

Two hundred thirty-one patients (119 men and 112 women) aged 34.4 ± 16.9 years (mean $\pm$ SD) with histories of atopic asthma, allergic rhinitis, atopic dermatitis, or a combination thereof were studied. All subjects had a history of physician-diagnosed atopic disease, and they received several months of minimal treatment for the given disease. Twenty-five of the 231 patients had atopic asthma (14 men and 11 women), 53 had allergic rhinitis (25 men and 28 women), 10 had atopic dermatitis (4 men and 6 women), 73 had both asthma and rhinitis (35 men and 38 women), 7 had asthma and dermatitis (3 men and 4 women), 7 had rhinitis and dermatitis (2 men and 5 women), and 56 had all 3 diseases (36 men and 20 women). The patients were recruited from the Department of Immunology, St Ann University Hospital, Brno, and from practicing allergologist surgical records (4 centers). All clinicians used the same criteria for diagnosis, inclusive of the same prick tests and the same criteria of pulmonary function testing for diagnosis, and each center followed a uniform protocol with regard to minimum criteria, allowing the comparison of data among centers. The main questionnaire collected information on upper and lower airways symptoms, description of asthma attacks, and/or symptoms of skin damage. The patients were also asked about seasonal variations, the frequency of the symptoms, and precipitants such as dust exposure, exercise, cold air, and infections. Detailed data on environmental quality were collected (active and passive smoking, indoor allergens, irritant exposure, and occupational exposures).

Asthma was defined according to American Thoracic Society criteria.³² The presence of asthma was documented by evaluation through history, physical examination, and the presence of reversible airways disease on pulmonary function testing. In our study bronchial hyperresponsiveness was not measured during the original evaluation. All patients were in the stable phase of disease in the course of the medical examination. Disease severity ranged from slight to severe status, with several patients requiring only

intermittent, seasonal therapy. The others were treated continuously, including systemic corticotherapy. Treatment resulted in normal values of pulmonary function tests. Because all patients meeting the criteria of our study were included, the distribution of studied disease severity in this study was not very different from that found in the general population in our country; the studied group was a representative sample of the Czech population.

Patients must have had the classic definitions of asthma and not represent unusual phenotypes that may or may not be labeled "asthmatic." Patients with asthma must meet the following criteria: (1) recurrent symptoms of cough, wheezing, and dyspnea; (2) history of a physician diagnosis of asthma and/or prior history of asthma therapy; and (3) the absence of exclusion criteria, consisting of items to exclude those disorders that cause airway obstruction. All patients had to satisfy all 3 criteria.

Atopic dermatitis was defined according to the major and minor diagnostic criteria for atopic dermatitis initially proposed by Hanifin and Rajka³³ in 1980—for diagnosis, at least 3 major criteria and at least 3 minor criteria must be present. Diagnosis of atopic dermatitis was determined by the specialist. These patients were first seen with a typical clinical appearance, a positive personal history for atopic disorders, positive skin prick test responses, and facultatively elevated IgE levels.

Subjects with allergic rhinitis had atopy and symptoms of hay fever confirmed by a specialist before entry into our study.

The diagnosis of atopy was made on the basis of several criteria, including clinical signs of atopic disease. Atopy was defined (widely used clinical criteria) by the presence of 1 or more of the following criteria. The first criterion was a positive skin prick test response (>3 mm than the negative control) to 1 or more of the following common antigens: house dust mite (*Dermatophagoides pteronyssinus*), common grass pollens and tree pollens, animal fur extracts (cat and dog dander), common molds (*Alternaria*, *Cladosporium*, and *Aspergillus* spp), histamine (as a positive control), and physiologic solution (as a negative control). We used standardized test procedures, and a set of commercially available allergens routinely used in practice in our country (Sevapharm, Czech Republic). The second criterion was a total serum IgE level above normal values (ie, more than 150 IU/mL in nonsmoking adults). Measurement of total serum IgE was performed by an immunoturbidimetric test (Boering). The total serum IgE levels in our atopic patients were 306 ± 349 IU/mL (mean $\pm$ SD). The third and final criterion was the presence of raised specific serum IgE levels (>0.35 kU/L, by ELISA [Biovendor]) to 1 or more of the following common antigens: *D pteronyssinus*, mixed epithelia (cat, dog, horse, and cow), mixed grasses, and mixed molds.

Control subjects and patients with clinical evidence of any cardiovascular disease were excluded because of a possible association between the I/D polymorphism of the ACE gene and some variants of the angiotensinogen gene with an increased risk of cardiovascular disease.

All subjects gave written informed consent to participation in the study. The study was approved by the Committee for the Ethics of Medical Experiments on Human Subjects, Medical Faculty, Masaryk University, Brno (No 64/93, 1993).

Detection of ACE gene polymorphism

The human ACE gene is located at chromosome 17q23. The 287-bp I/D polymorphism is located in intron 16 of the ACE gene. The insertion itself corresponds to 1 alu repetitive sequence, which is not present at the D allele.

Genomic DNA from the subjects was prepared from peripheral blood leukocytes by standard procedures. A 287-bp I/D polymorphism in intron 16 of the ACE gene was examined by PCR,³⁴ with a subsequent verification of the DD genotype by the method of

Shanmugam et al.³⁵ The PCR products were separated by electrophoresis on 2% agarose gel and identified with ethidium bromide staining. The polymorphism detected by PCR was characterized by a 490-bp product in the presence of the insertion and as a 190-bp fragment in the absence of the insertion, which results in 3 different genotypes: DD and II homozygotes and ID heterozygotes.⁹

Determination of missense mutation with methionine to threonine amino acid substitution at codon 235 in the angiotensinogen gene

The human angiotensinogen gene is located on chromosome 1q42-43. The T→C transition at nucleotide 704 in exon 2 leads to the substitution of threonine (T) for methionine (M) at amino acid position 235. The threonine allele was associated with the elevated plasma levels of angiotensinogen.

Polymorphism M235T of the angiotensinogen gene was detected by the method of Russ et al.³⁶ PCR products were digested with *AspI* at 37°C for 3 hours. The digested fragments were separated by electrophoresis in 2.5% agarose gel and visualized with ethidium bromide. The homozygous methionine allele of the angiotensinogen gene (M235) appears as a nondigested single 165-bp band and the T235 genotype as digested 141- and 24-bp bands. Heterozygotes had all 3 bands (ie, 165, 141, and 24 bp).

Determination of point mutation T/C ET-1 in intron 4, position 8000

The human ET-1 gene is located on chromosome 6p23-24. Taq polymorphism of the ET-1 gene in intron 4, position 8000, either contains a target sequence for TaqI-restricting enzyme or the allele loses this cleavage site.

Taq polymorphism of the ET-1 gene (intron 4, position 8000) was detected by using our original PCR method. The PCR product of the length of 358 bp (primers 5'-CAA ACC GAT GTC CTC TGT A-3' and 5'-ACC AAA CAC ATT TCC CTA TT-3') in its nonmutated form contains a target sequence for TaqI-restrictive enzyme, whereas a mutated product loses this cleavage site. Restriction analysis with enzyme TaqI and electrophoretic separation in 2.5% agarose gel stained with ethidium bromide is then followed by evaluating the genotype of the individual (homozygote [++], 150 and 208 bp; heterozygote [+–], 150, 208, and 358 bp; and homozygote [––], 358 bp).

To assess the statistical structure of the set (namely the randomness and mutual independence of the occurrence of alleles) and to detect the presence of possible interactions between the genes, we used tests for Hardy-Weinberg equilibrium, as well as those for mutual independence of the allelic combinations (applying the chi-squared test). The Fisher exact test was applied to evaluate differences between the groups in the occurrence of the single alleles in all 3 genes. Program package Statistica v 3.0 (Statsoft Inc) was used.

RESULTS

For our analysis, subjects were organized in the following 9 groups: healthy control subjects, asthmatic patients, nonasthmatic patients, patients with rhinitis, patients without rhinitis, patients with eczema, patients without eczema, patients with both asthma and rhinitis, and patients without both asthma and rhinitis.

The genotype distribution in all 9 groups for all 3 polymorphisms (27 combinations) was within the Hardy-Weinberg equilibrium.

The results are summarized in Tables I to IV. Regarding the fact that a multiple comparison is involved, only *P* values less than .05 after Holm's procedures are listed as significant (in bold letters).

The significant shifts (Table I) in the allelic frequencies in the I/D ACE and angiotensinogen gene polymorphisms occurred in the patients with atopic disease compared with healthy control subjects (*P* = .0025 and *P* = .0204, respectively). No significant difference in the allelic frequencies was found for T→C transition in intron 4, position 8000, in the ET-1 gene, having compared all atopic patients with the control subjects (*P* = .1774, not significant).

The differences in the I/D ACE and the M235T angiotensinogen genotypes between single subgroups of atopic diseases were not significant, and they are caused by differences in single-size groups (Tables II and III).

Although no significant differences in allelic frequency of ET-1 polymorphism between patients and control subjects was found, significant differences were found among single types of atopic diseases (Table IV). The difference has high significance between patients with both asthma and rhinitis and patients without both respiratory manifestations. In detailed analysis the presence of the (–) allele is more frequent in asthmatic patients, as well as in patients with rhinitis, and it is most frequent in patients with a combination of both respiratory diseases (although the frequency of the [–] allele is indistinguishable from that of healthy control subjects). The lower significance of difference proved between asthmatic and nonasthmatic patients and between patients with and without rhinitis is influenced by lower numbers of subjects in groups of patients without either asthma or rhinitis.

Detailed analysis of a mutual coincidence of the alleles done by examining each pair of genes did not reveal any deviations from their random combining in any of the groups. The results of this analysis indicate an absence of any serious role of mutual interactions of the examined mutations in the occurrence of the diseases in the study.

DISCUSSION

Our results confirm originally published data on the association of the DD genotype of the ACE gene with asthma; the first reported evidence for an association between the I/D polymorphism of the ACE gene and asthma was published in 1997 by Benessiano et al.³⁷ In addition, we demonstrated a significant difference in the frequency of the D allele itself between healthy and atopic persons. Detailed analyses showed that shift in this direction was observed in all subgroups independently on different clinical manifestation. Hypothetically, the high ACE levels in the plasma and the tissues associated with the D allele, and eventually with the DD genotype, could be expected to have a considerable influence on the pathogenesis of the atopic disease, either because of its effect on the smooth muscle of the bronchi

TABLE I. Genotype distribution and allele frequencies of ACE, angiotensinogen, and ET-1 genes

	Healthy control subjects (n = 141)	All atopic patients (n = 231)
II	37	40
ID	75	114
DD	29	77
I allele	0.528	0.420
D allele	0.472	0.580
	<i>P</i> = .0025	
MM	35	80
MT	75	112
TT	31	37
M allele	0.514	0.594
T allele	0.486	0.406
	<i>P</i> = .0204	
++	85	157
+-	44	64
--	5	7
+ allele	0.799	0.829
- allele	0.201	0.171
	<i>P</i> = .1774	

P values in bold are significant.

by activating angiotensin II, or on proinflammatory cells by releasing various mediators of inflammation or bronchoconstriction from the cells. This influence is probably easier to demonstrate in asthmatic patients than in nonasthmatic atopic individuals because ACE is heavily expressed in the lungs.³⁸

ACE has been identified as a membrane-bound enzyme in several types of cells, including vascular endothelial cells, various absorptive epithelial cells, neurons, and macrophages. It is also present in a circulating form in biologic fluid such as plasma. In addition to the well-established circulating (endocrine) renin-angiotensin system, cellular (autocrine) renin-angiotensin systems have been described.³⁹⁻⁴¹ The biologic function of ACE in T lymphocytes, as well as in macrophages, remains largely unknown. ACE transcription may be activated during T-cell differentiation, or conversely it may be suppressed in mature B lymphocytes, but few B cells are present in the bronchi of asthmatic subjects. The ACE in mononuclear cells may participate in local production or degradation of regulatory peptides (eg, at the site of an inflammation reaction). Atopic diseases are accompanied by increased numbers of mast cells, activated eosinophils, and CD4-positive cells with a T_{H2} functional phenotype, with their altered distribution and increased releasability. The renin-angiotensin system itself is likely to influence cytokine synthesis. This is supported by a study demonstrating induction of platelet-derived growth factor by angiotensin II in smooth muscle cells.^{42,43} IL-1-induced cytokine synthesis was increased by approximately 20% in the presence of angiotensin II.

Angiotensinogen, a precursor of angiotensin I, is produced by hepatocytes and other cells. Its transcription is

TABLE II. ACE genotype distribution and allele frequencies

	Healthy control subjects (n = 141)	Asthmatic patients (n = 161)	Non-asthmatic atopic patients (n = 70)	Patients with rhinitis (n = 189)	Atopic patients without rhinitis (n = 42)	Patients with eczema (n = 80)	Atopic patients without eczema (n = 151)	Patients with both asthma and rhinitis (n = 129)	Patients without both asthma and rhinitis (n = 102)
Genotype distribution									
II	37	23	17	34	6	14	26	18	22
ID	75	85	29	94	20	40	74	70	44
DD	29	53	24	61	16	26	51	41	36
Allelic frequency									
I allele	0.528	0.407	0.450	0.429	0.381	0.425	0.417	0.411	0.431
D allele	0.472	0.593	0.550	0.571	0.619	0.575	0.583	0.589	0.569
P values*	NS	NS	NS	NS					

NS, Not significant.

*Probability of difference between atopic groups.

TABLE III. Angiotensinogen M235T genotype distribution and allele frequencies

	Healthy control subjects (n = 141)	Asthmatic patients (n = 161)	Non-asthmatic atopic patients (n = 68)	Patients with rhinitis (n = 187)	Atopic patients without rhinitis (n = 42)	Patients with eczema (n = 80)	Atopic patients without eczema (n = 149)	Patients with both asthma and rhinitis (n = 129)	Patients without both asthma and rhinitis (n = 100)
Genotype distribution									
MM	35	56	24	68	12	33	47	48	32
MT	75	77	35	90	22	32	80	59	53
TT	31	28	9	29	8	15	22	22	15
Allelic frequency									
M allele	0.514	0.587	0.610	0.604	0.548	0.612	0.584	0.601	0.585
T allele	0.486	0.413	0.390	0.396	0.452	0.388	0.416	0.399	0.415
P values*			NS		NS		NS		NS

NS, Not significant.

*Probability of difference between atopic groups.

TABLE IV. ET-1 genotype distribution and allele frequencies

	Healthy control subjects (n = 134)	Asthmatic patients (n = 161)	Non-asthmatic atopic patients (n = 69)	Patients with rhinitis (n = 187)	Atopic patients without rhinitis (n = 41)	Patients with eczema (n = 80)	Atopic patients without eczema (n = 148)	Patients with both asthma and rhinitis (n = 129)	Patients without both asthma and rhinitis (n = 100)
Genotype distribution									
++	85	105	54	125	32	58	99	79	78
+-	44	50	14	55	9	18	46	43	21
--	5	6	1	7	0	4	3	6	1
Allelic frequency									
+ allele	0.799	0.807	0.884	0.816	0.890	0.838	0.824	0.785	0.885
- allele	0.201	0.193	0.116	0.184	0.110	0.162	0.176	0.215	0.115
P values*			.0248		.0670		NS		.0033

P value in bold is significant.

NS, Not significant.

*Probability of difference between atopic groups.

actually responsive to 3 known mediators of the acute-phase response (ie, glucocorticoids and 2 cytokines, IL-1 and TNF- α).¹³⁻¹⁵ The gene expression is greatly subjected to tissue-specific hormonal and developmental control. We studied polymorphism M235T of the angiotensinogen gene, which is correlated with plasma angiotensinogen levels.⁴⁴ It is interesting to note that atopic patients show a higher M allele frequency compared with the control group. The prevalence of MM genotype with the lowest angiotensinogen levels as a substrate, and conversely the prevalence of the D allele (and DD genotype) of the ACE gene with the highest activities of the enzyme, may lead to differences in the kinetics of the enzyme-substrate reaction.

In the vessel wall ET-1 can be released by angiotensin II.^{22,45,46} ET-1 is a potent vasoconstrictor agent with pressor and growth-promoting actions,⁴⁷ and it acts primarily as a local hormone^{48,49} that can function as a proinflammatory peptide. Macrophages are a source of ET secretion,⁵⁰ and although polymorphonuclear lymphocytes fail to synthesize ET, they rapidly convert exogenous pro-ET to bioactive ET.⁵¹ At the sites of inflammation, both these cells are present and likely to increase the local concentration of ET. In patients without both respiratory manifestations, a significant decrease in the frequency of the (–) allele was found compared with patients with both asthma and rhinitis at T→C transition in intron 4 of the ET-1 gene. Simultaneously, the allele's frequency in atopic patients with both asthma and rhinitis does not differ from that found in healthy volunteers. Considering the lack of published data on the relationship of this polymorphism to the plasmatric or local levels of ET-1, it is not possible to discuss the relationship of the polymorphism and the levels to the etiopathogenesis of the atopic phenotype.

Our results would be confirmed by a larger case-control study with other investigators if these genes would be included in the genetic background of atopic diseases.

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