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Original Article

Association between angiotensin-converting enzyme gene polymorphism and childhood allergic rhinitis in Taiwan

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Background and Purpose: Angiotensin-converting enzyme (ACE) play a role in inactivating bradykinin and tachykinins. Bradykinin and tachykinins are potent mediators of inflammatory reaction. An insertion/deletion polymorphism of the ACE gene has been shown to be associated with serum ACE levels. We hypothesized that ACE polymorphism might play a role in allergic rhinitis development.

Methods: Seventy five children aged 6-13 years with atopic allergic rhinitis and 66 age- and gender-matched healthy children were studied. ACE genotypes were determined by polymerase chain reaction. Serum total immunoglobulin E (IgE) and allergen-specific IgE levels were also measured.

Results: The frequencies of the DD and non-DD genotypes, and of the II and non-II genotypes did not differ significantly between healthy children and allergic rhinitis children (chi-squared test, p=1.000 and 0.438, respectively). There was no association of ACE genotype and mean IgE levels in rhinitis children or healthy controls.

Conclusion: The results of our study indicate that polymorphism of the ACE gene is unrelated to the development of allergic rhinitis, the duration of allergic rhinitis, serum IgE levels, and allergen-specific IgE in Taiwanese children.

Key words: Genetic polymorphism, genotype, peptidyl-dipeptidase A, perennial allergic rhinitis, Taiwan

Introduction

Atopic diseases including asthma, allergic rhinitis, and atopic dermatitis represent the development of immediate hypersensitivity reactions to environmental antigens. These diseases are complex genetic disorders involving multiple interactions from numerous genes combined with environmental influences. Allergic rhinitis is a hyper-responsive reaction of the nasal mucous membranes to a variety of allergens. The cellular and humoral aspects of the inflammation have previously been studied [1].

Angiotensin-converting enzyme (ACE), a membrane-bound ectoenzyme, is found on the surfaces of vascular endothelial cells, renal epithelial cells, lymphocytes, and macrophages, and also circulates in the plasma. One function of ACE is to inactivate naturally occurring proinflammatory peptides such as bradykinin and substance P [2]. Thus, ACE exerts an anti-inflammatory effect. Bradykinin and substance P are believed to play important roles in the pathogenesis of atopic disease, especially in neurogenic inflammation [3].

The ACE gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) of a nonsense DNA fragment [4]. The polymorphism is located in intron 16. Thus, there are 3 genotypes: DD, ID, and II. Studies show that genotypes of the ACE gene are correlated with different plasma and cellular ACE concentrations [5,6]. The DD genotype is associated with the highest plasma levels, while the II genotype correlates with the lowest levels.

From the above studies, we know that ACE polymorphism plays a role in atopic diseases including allergic rhinitis. To date, however, the role of ACE

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gene polymorphism in allergic rhinitis is inconclusive [7]. We therefore examined genes of allergic rhinitis patients and controls to determine whether any association exists.

Methods

Study population

Seventy five unrelated children with allergic rhinitis were recruited from the pediatric outpatient clinic of a university teaching hospital in Taichung, Taiwan. Forty two of them had had allergic rhinitis for more than 2 years, while the others had had it for less than 2 years. The diagnosis of allergic rhinitis was based on clinical grounds by 2 specialists as the occurrence of allergic rhinitis symptoms and signs including sneezing, watery rhinorrhea, and blocked or itchy nose in the absence of a common cold during the previous 12 months. Subjects with signs and symptoms of asthma, or a history of asthma were excluded. The criteria of atopy included clinical signs, a positive family history, and abnormal serum total immunoglobulin E (IgE) or at least 1 allergen-specific IgE measurement >0.35 kU/L (described below). In the controls, 66 age- and gender-matched unrelated healthy subjects were randomly recruited from 3 elementary schools in the same area of Taiwan. None of these children, their brothers and sisters, or their parents had any history of atopic disease. Serum IgE levels of these healthy children were within a normal range (<150 kU/L).

The studies were arranged during September to December 2004. Control subjects and patients who had evidence of cardiovascular, renal, pulmonary or nasal disease other than allergic rhinitis were excluded. All subjects were from the same area of west central Taiwan, and were of the same race (ethnic Han Chinese). Informed consent was obtained from their parents and protocols were approved by the ethics committee at our institution.

Total and allergen-specific IgE

Total serum IgE levels were tested by a fluoresence enzyme immunoassay using a commercially available CAP system (Pharmacia, Uppsala, Sweden). Concentrations of specific IgE reactions to common aeroallergens in Taiwan, including *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *Dermatophagoides microcerus*, *Blatella germanica*, dog dander and cat dander were also tested by a fluoresence enzyme immunoassay (CAP system; Pharmacia).

Determination of the ACE genotype

The D and I alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the ACE gene, as previously described [6]. Genomic DNA was extracted from the peripheral blood using standard procedures [8]. PCR products were separated by 2% agarose gel electrophoresis, and identified with ethidium bromide staining. Subjects were then classified according to the presence or absence of the 287-bp insertion in intron 16 of the ACE gene. Subjects were then separated into the DD, ID, or II genotype.

Statistical analysis

The frequencies of the ACE genotypes in allergic rhinitis and control groups were compared using 2-tailed chi-squared test and Fisher's exact test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated as a measure to evaluate the association of the ACE genotype with allergic rhinitis. A 2-sided p value of <0.05 was considered significant.

Data are expressed as the mean ± standard deviation; t test and analysis of variance were used to study the relationships of genotypes and serum IgE levels. As the distributions of the serum total IgE levels were skewed, they were log-transformed to approximate a normal distribution.

Results

Frequencies of alleles and genotypes

The demographic data of subjects are shown in Table 1. There was no difference in gender distribution and age between the 2 groups. Among the healthy children, the frequencies of the I and D alleles were 0.75 and 0.25, respectively. Distribution of the II, ID, and DD genotypes was 54.5%, 41.0% and 4.5%; that of the DD and non-DD genotypes was 4.5% and 95.5%; and that of the II and non-II genotypes was 54.5% and 45.5%, respectively (Table 2).

Among children with allergic rhinitis, the frequencies of the I and D alleles were 0.71 and 0.29,

Table 1. Demographic data of subjects

	No.	Gender (F/M)	Mean age (years ± SD)
Allergic rhinitis patients	75	50/25	8.83 ± 2.08
Controls	66	43/23	9.20 ± 1.59
p (difference between groups)		0.242	0.850

Abbreviations: F = female; M = male; SD = standard deviation

Table 2. Genotype and allele frequencies of angiotensin-converting enzyme (ACE) gene polymorphism

ACE genotypes and alleles	Controls (n = 66) No. (%)	Allergic rhinitis patients (n = 75) No. (%)
DD[a]	3 (4.5)	4 (5.3)
Non-DD (ID+II)[a]	63 (95.5)	71 (94.7)
II[b]	36 (54.5)	36 (48.0)
Non-II (ID+DD)[b]	30 (45.5)	39 (52.0)
D	33 (25.0)	43 (29.0)
I	99 (75.0)	107 (71.0)

[a]Comparison of frequencies of DD and non-DD genotype, p=1.000 (Fisher's exact test); odds ratio = 1.183; 95% confidence interval, 0.255-5.491.

[b]Comparison of frequencies of II and non-II genotype, p=0.438 (chi-squared test); odds ratio = 0.769; 95% confidence interval, 0.396-1.493.

respectively. Distribution of the II, ID, and DD genotypes was 48.0%, 46.7%, and 5.3%; that of the DD and non-DD genotypes was 5.3% and 94.7%; and that of the II and non-II genotype was 48% and 52%, respectively (Table 2).

The frequencies of the I and D alleles did not differ significantly between healthy children and those with allergic rhinitis children (chi-squared, p=0.495). Also, the frequencies of the DD and non-DD genotypes, and of the II and non-II genotypes between healthy children and allergic rhinitis children did not differ significantly (chi-squared, p=1.000 and 0.438, respectively). Among the children with allergic rhinitis, the frequency of the DD genotype was higher, but without statistical significance (OR, 1.183; 95% CI, 0.255-5.491). Among the children with allergic rhinitis, the frequency of the II genotype was lower, but without statistical significance (OR, 0.769; 95% CI, 0.396-1.493) [Table 2]. Among the children with allergic rhinitis for more than 2 years, the frequencies of the genotypes did not differ from those of children with allergic rhinitis for less than 2 years (Table 3).

Serum total IgE and allergen-specific IgE

Because the distribution of serum total IgE levels was skewed, it was log-transformed to approximate a normal distribution. Table 4 describes the association between mean IgElog and ACE genotypes. There was no association of the ACE genotype and mean IgElog in allergic rhinitis children (p=0.1) or healthy children (p=0.429). Table 5 describes the association of allergen-specific IgE and genotypes in rhinitis children; no association was noted.

Discussion

ACE is a membrane-bound ectoenzyme. It is located on the surface of vascular endothelial cells, renal epithelial cells, lymphocytes, and macrophages, and also circulates in the plasma. The insertion/deletion polymorphism within intron 16 of the ACE gene has been shown to be associated with circulating ACE levels [4]. Serum ACE levels were higher in subjects with the DD genotype, and lower in subjects with the II genotype. A study by Suehiro et al showed that the expression of ACE mRNA formation from the D allele is higher than that from the I allele [9].

ACE plays a key role in converting angiotensin I to angiotensin II, and in inactivating tachykinins and bradykinin [2]. Angiotensin II causes broncho-constriction by directly affecting airway smooth muscles and by other mechanisms [10]. The level of angiotensin II is increased in acute asthma. Tachykinins and bradykinin are inflammatory mediators. They induce bronchial edema, extravasation of plasma and mucus

Table 3. Genotype and allele frequencies in children with allergic rhinitis for more or less than 2 years

ACE genotypes and alleles	Allergic rhinitis >2 years (n = 42) No. (%)	Allergic rhinitis <2 years (n = 33) No. (%)
DD[a]	2 (4.8)	2 (6.0)
Non-DD (ID+II)[a]	40 (95.2)	31 (94.0)
II[b]	17 (40.4)	16 (48.5)
Non-II (ID+DD)[b]	25 (59.5)	17 (51.5)
D[c]	27 (32.1)	19 (28.7)
I[c]	57 (67.8)	47 (71.2)

Abbreviation: ACE = angiotensin-converting enzyme

[a]Comparison of frequencies of DD and non-DD genotype, p=1.000 (Fisher's exact test).

[b]Comparison of frequencies of II and non-II genotype, p=0.488 (chi-squared test).

[c]Comparison of frequencies of D and I allele, p=0.658 (chi-squared test).

Table 4. Serum immunoglobulin Elog levels and angiotensin-converting enzyme genotypes (mean ± standard deviation)

Genotype	Controls	Allergic rhinitis patients
II	1.42 ± 0.35 (n = 36)	2.6 ± 0.45 (n = 36)
ID	1.42 ± 0.42 (n = 27)	2.34 ± 0.60 (n = 35)
DD	1.71 ± 0.15 (n = 3)	2.24 ± 0.67 (n = 4)
p	0.429	0.100

hypersecretion [11]. Several studies of the relationship between asthma and ACE polymorphism indicated that the frequencies of the DD genotype were higher in asthma patients [7,12,13]. Benessiano et al [12] suspected that angiotensin II formation plays a major role in asthma formation.

Lurie et al [14] found that in patients with allergic rhinitis, ACE plays a role in the degradation of substance P in the nasal cavity. Gotoh [15] found that in allergic rhinitis patients, premedication with an ACE inhibitor enhanced the action of bradykinin in inducing an increase in nasal mucosa permeability. Because there are no smooth muscles in the nasal cavity and from the studies mentioned above, we hypothesized that the inactivation of bradykinin and tachykinins in the nasal cavity by ACE might play a major role in allergic rhinitis formation. Therefore, the II genotype (with the lowest serum ACE level) may be a risk factor for allergic rhinitis formation due to the lower ability to inactivate bradykinin and tachykinins. But study results from Holla et al [7] indicated that in allergic rhinitis subjects, the frequency of the DD genotype was higher. Kim et al [16] reported that ACE gene polymorphism was unrelated to the development of allergic rhinitis in a Korean population. That finding is not compatible with our hypothesis. Therefore, we conducted this study to evaluate whether the II or DD genotype of the ACE polymorphism influences allergic rhinitis formation.

In our study, we found no significant associations between ACE gene polymorphism and allergic rhinitis or its duration. The frequency of the II genotype in allergic rhinitis patients was lower than that of healthy children, but the difference was not statistically significant (chi-squared test, p=0.438). This result did not fit our hypothesis. The frequency of the DD genotype in allergic rhinitis patients was slightly higher than that of healthy children, but the difference was not statistically significant (chi-squared test, p=0.100). The result was the same as that from the study by Kim et al [16], but different from that of a study by Holla et al [7].

Table 5. Allergen-specific immunoglobulin Elog levels and angiotensin-converting enzyme genotype in children with allergic rhinitis

Allergen sensitivity	DD + ID (%)	II (%)	*p*
Blomia tropicalis	67.7	70.6	0.664
Dermatophagoides pteronyssinus	60.0	64.7	0.687
Dermatophagoides farinae	60.0	67.6	0.509
Blatella germanica	25.7	23.5	0.833
Cat dander or dog dander	5.7	20.6	0.084

Several factors could account for the differences between our hypothesis and our study results. First, although a relationship between ACE polymorphism and serum ACE levels has been reported [4,9,17], and an increase of ACE activity in the nasal cavity in subjects with allergic rhinitis has been noted, the link between ACE polymorphism and nasal ACE activity has not been directly proven. No study has examined the relationship between ACE polymorphism and nasal cavity ACE activity. Future study is necessary to determine that relationship. Second, a review article by Kaplan et al [18] indicated that although bradykinin can produce hyperemia, rhinorrhea, and nasal congestion, it has not been possible to assess its contribution to symptoms, because a potent and selective bradykinin receptor antagonist that can be administered in vivo has not been available. Therefore, the lack of a contribution by bradykinin to the symptoms of allergic rhinitis might explain our study results. Further study is necessary.

We suggest several possible causes for the differences between our study results and those of Holla et al [7]. First, ethnic backgrounds may have been a factor. Second, the study subjects from Holla et al were adults, but in our studies, study subjects were all children aged 6-13 years. Age may have been a factor influencing the study results. Third, in our study, none of the children in the control group had any family history of atopic disease, whereas family atopic disease histories were not considered by Holla et al [7]. Fourth, in our study, all children with allergic rhinitis were atopic, but rhinitis subjects from the study of Holla et al [7] were not.

In summary, the results of our study indicate that polymorphism of the ACE gene is unrelated to the development of allergic rhinitis, the duration of allergic rhinitis, serum IgE levels, and allergen-specific IgE in Taiwanese children.

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