

ACE gene polymorphism might disclose why some Taiwanese children with allergic rhinitis develop asthma symptoms but others do not

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Although allergic asthma and allergic rhinitis have recently been considered to be a single disease, many questions remain unanswered. Why do some atopic patients develop asthma symptoms and others develop allergic rhinitis symptoms? Which factors play a role in the development of different allergic phenotypes? We hypothesized that angiotensin-converting enzyme (ACE) gene polymorphism might play a role in the development of asthma phenotypes in children with allergic rhinitis. The study sample included 106 children with allergic rhinitis, but no asthma, and 105 age- and gender-matched children with allergic rhinitis and asthma. Subjects of both groups exhibited the same systemic immunologic changes and allergen sensitivities. Controls consisted of 102 healthy children. The ACE genotype was determined by polymerase chain reaction. The serum total immunoglobulin E (IgE) level, allergen-specific IgE sensitivity, and eosinophil count were also measured. The frequencies of the DD genotype were significantly higher in the children with both allergic rhinitis and asthma than in the children with allergic rhinitis but no asthma [$p = 0.018$; odds ratio (OR) = 3.257; (1.222–8.680)]. Results of this study suggest that ACE gene polymorphism DD genotype might play a role in the development of the asthma phenotype in children with allergic rhinitis.

Ko-Huang Lue^{1,2}, Min-Sho Ku¹, Ching Li³, Hai-Lun Sun^{1,2}, Hong-Shen Lee⁴ and Ming-Chih Chou²

¹Division of Allergy, Asthma and Rheumatology, Department of Pediatrics, Chung Shan Medical University Hospital, ²Institute of Medicine and Departments of ³Microbiology and Immunology and ⁴Public Health, Chung Shan Medical University, Taichung, Taiwan

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Ko-Huang Lue MD, Division of Allergy, Asthma and Rheumatology, Department of pediatrics, Chung Shan Medical University Hospital. No. 110, Sec. 1, Chien-Kuo N. Road, Taichung, Taiwan 402, R.O.C.

Tel.: +886-4-24739595

Fax: +886-4-24710934

E-mail: cshy095@csh.org.tw

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Atopy is the inherited propensity to respond immunologically to common naturally occurring allergens with continual production of immunoglobulin E (IgE). Asthma and allergic rhinitis are two types of atopic disease. The two diseases are currently thought of as a continuum of inflammation involving a single common pathway (1), or one airway, one disease (2). To date, data point toward a systemic link between the upper and lower airways (3). Although allergic asthma and allergic rhinitis have recently been considered a single disease, many questions remain unanswered. Why do some allergic rhinitis patients develop asthma symptoms but others do not? Which factors play a role in the development of different allergic phenotypes?

Studies have indicated that local allergen stimulation can induce systemic immunologic changes (3–5) in atopic patients. Under such systemic immunologic changes, some factors might induce asthmatic symptoms and signs (S/S), whereas others might induce allergic rhinitis S/S. To find such factors, we recruited subjects with the same atopic state and systemic immunologic changes, and attempted to rule out other confounding factors. If we could find a factor that is noted in subjects with asthma, but not in subjects without asthma, then such a factor might play a role in the formation of different phenotypes.

Atopic disease is a complex disorder in which interactions from numerous genes are combined

with environmental influences. Studying genetic factors is one method to elucidate the biological mechanism of atopic disease. Angiotensin-converting enzyme (ACE), an ectoenzyme, plays a key role in converting angiotensin I to angiotensin II. Angiotensin II is a potent airway smooth muscle contractile agonist (6). There is smooth muscle in the bronchioles, but none in the nasal cavity. Previous studies indicated that in ACE gene polymorphism, the DD genotype is associated with asthma (7–10) and higher serum ACE levels (9, 11). We hypothesized that in patients with allergic rhinitis, ACE might play a role in formation of the asthma phenotype. If the frequency of the ACE gene DD genotype is higher in subjects with asthma and lower in subjects without asthma, our hypothesis would be supported.

Materials and methods

Study population

Group 1: the allergic rhinitis without asthma group. One hundred and eleven unrelated children aged 8–13 yr (mean age 9.97 ± 1.78 y/o; male/female 63.8/36.2) with allergic rhinitis were recruited from the pediatric outpatient clinics of a university teaching hospital in Taichung, Taiwan. No asthma S/S, no abnormal pulmonary function (abnormal FEV1 and 15% or greater reversibility of FEV1 after bronchodilator treatment) were found. The diagnosis of allergic rhinitis by two immunologists was based on clinical grounds, as the occurrence of allergic rhinitis S/S including sneezing, watery rhinorrhea, and blocked or itchy nose for 2 yr in the absence of a common cold. After 2-yr follow-up, five subjects with asthma S/S or abnormal pulmonary function but the diagnosis of asthma was in doubt were excluded from our study. Total 106 children were enrolled in this study.

Group 2: the allergic rhinitis with asthma group. One hundred and five age- and gender-matched children (mean age 9.91 ± 1.62 y/o; male/female 68.9/31.1) with allergic rhinitis combined with asthma were recruited from the same hospital. These children had an allergic rhinitis history for 2 yr. Asthma had been noted at the same time of the onset of allergic rhinitis. Asthma was diagnosed by two immunologists and fit the criteria of the American Thoracic Society (12). The diagnosis of asthma was confirmed by a clear clinical history with clinical symptoms (episodic wheezing, chest tightness, and dyspnea), 15% or greater reversibility of

FEV1 after bronchodilator treatment and reversibility of the wheezing and dyspnea after bronchodilator treatment.

All children in the two groups fit the criteria of atopy, and those with rhinitis or asthma caused by etiologies other than from allergic reactions were excluded. The criteria of atopy included S/S, a positive family history, and at least one allergen-specific IgE of >0.35 kU/l (described below).

Group 3: the control group. One hundred and two age-matched (mean age 9.94 ± 1.32 y/o; male/female 49.0/51.0), unrelated healthy children were randomly recruited from three elementary schools in the same area of Taiwan. Neither these children, their brothers/sisters, nor their parents had any atopic disease. Serum IgE levels, eosinophil counts, allergen-specific IgE values (<0.35 kU/l) and pulmonary function (FEV1) with bronchodilator tests, were all within normal ranges. Subjects who had evidence of cardiovascular, renal, pulmonary, or nasal disease other than allergic rhinitis or asthma were excluded.

All subjects were from the same area of west-central Taiwan, and were of the same race. Informed consent for participation in the study was obtained from the parents of all subjects. The study protocols were approved by the Ethics Committee of the Hospital.

Total and allergen-specific IgE levels

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

Blood eosinophil count

A peripheral venous blood sample was collected, and the eosinophils were counted in a Fuchs Rosenthal chamber after staining with eosinophil staining solution.

Determination of the ACE genotype

Genomic DNA was extracted from the peripheral blood using standard procedures

(13). 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 (14). The sequence of the sense primer and the anti-sense primer were 5'-CTG-GAGACCACT CCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-3', respectively. After heating 94°C for 3 min, the DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. A final extension for 5 min at 72°C was included. 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, and grouped according to the DD, ID, or II genotype.

Statistical analysis

The frequencies of the ACE genotypes in children of Groups 1 and 2, and the controls were compared using two-tailed chi-square test. Logistic regression analysis with odds ratio was used to compare the association between different genotypes and different phenotypes, adjusting for the potential confounder's age and gender. The chi-square test was used for differences in prevalence of allergen sensitivity between Groups 1 and 2. Independent *t*-test was used to study the relationship between the three groups and serum eosinophil counts. Because the concentrations of IgE antibodies were not normally distributed, unpaired analyses were performed with the Mann-Whitney *U*-test to study the relationship between the three groups and serum IgE. Statistical analyses were undertaken using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). $p < 0.05$ were considered to be significant.

Results

Serum total and allergen-specific IgE levels, and eosinophil counts

Table 1 shows the mean serum IgE levels and mean eosinophil counts in the different groups. Parameter values between Groups 1 and 2 did not differ ($p = 0.483$ for total serum IgE levels and 0.954 for eosinophil counts). The parameters differed between the study sample and controls ($p < 0.05$). This means that subjects in Groups 1 and 2 had systemic immunologic changes and that these changes were of the same magnitude.

Table 2 shows the association of allergen-specific IgE levels in Groups 1 and 2. The distribution of allergen-specific IgE in the two groups did not differ ($p > 0.05$). This means that the allergen sensitivities of Groups 1 and 2 were almost the same.

Frequencies of alleles and genotypes

There was no difference in the age and gender distributions between Groups 1 and 2. In Group 1 (allergic rhinitis without asthma), the frequencies of the II, ID, and DD genotypes were 49.1%, 45.3%, and 5.7%, respectively. The distribution of the DD and non-DD genotypes was 5.7% and 94.3%. In Group 2 (allergic rhinitis with asthma), the frequencies of the II, ID, and DD genotypes were 45.7%, 38.1%, and

Table 1. Total serum IgE, Eosinophil count between groups

	Group 1 AR	Group 2 AR + AS	p-value
Total serum IgE (kU/l)	544.4 ± 533.5	598.4 ± 552.3	0.483
Eosinophil count (/μl)	486.7 ± 332.9	484.1 ± 280.7	0.954

Independent *t*-test was used for serum eosinophil counts. Because immunoglobulin E (IgE) concentrations were not normally distributed, Mann-Whitney *U*-test was used.

AR, allergic rhinitis; AS, asthma.

Table 2. Allergen specific IgE in Groups 1 and 2

	Group 1 AR (sensitive/total)	Group 2 AR + AS (sensitive/total)	p-value
<i>Blomia tropicalis</i> (Bt)	65% (69/106)	70% (74/105)	0.403
<i>Dermatophagoides pteronyssinus</i> (Dp)	63% (67/106)	71% (75/105)	0.203
<i>Dermatophagoides farinae</i> (Df)	64% (68/106)	70% (74/105)	0.327
<i>Blatella germanica</i> (Bg)	23% (24/106)	21% (22/105)	0.766
Cat or dog	13% (14/106)	19% (20/105)	0.249

The chi-square test was used for differences in prevalence of allergen sensitivity between the Groups 1 and 2.

AR, allergic rhinitis; AS, asthma; IgE, immunoglobulin E.

16.2%, respectively. The distribution of the DD and non-DD genotypes was 16.2% and 83.8%. In Group 3 (the control group), the frequencies of the II, ID, and DD genotype were 54.9%, 41.2%, and 3.9%, respectively (Table 3).

The frequencies of the II, ID, and DD genotypes between Groups 1 and 2 significantly differed ($p = 0.046$). The frequency of DD genotype between Groups 1 and 2 significantly differed ($p = 0.018$, odds ratio = 3.257; 95% CI, 1.222–8.680). In allergic rhinitis subjects with asthma (Group 2), the frequency of the DD genotype was higher than those without asthma (Group 1). It means that in children with allergic rhinitis, those with DD genotype were 3.257 times more likely to have asthma than those with ID and II genotypes. The frequencies of the II, ID, and DD genotypes between Group 1 and the controls did not significantly differ ($p = 0.647$) (Table 3), but did significantly differ between Group 2 and the controls ($p = 0.013$).

Discussion

The historic perspective of allergic rhinitis and asthma as distinct and separate entities is being displaced by current thinking that they are better described as a continuum of inflammation involving a single common pathway (1), or one airway, one disease (2). To date, data point toward a systemic link between the upper and lower airways (3). Systemic production of allergen-specific IgE, eosinophils, and IL-5 after local allergic sensitization was found by other studies (4, 5). Therefore, local allergen stimulation induces systemic immunologic changes including increases in serum IgE and IL-5 levels, and the

eosinophil count. In subjects with such systemic immunologic changes, some factors must determine the formation of the asthma phenotype, while other factors must determine the formation of the rhinitis phenotype. However, the factors have so far not been clearly elucidated. Our study tried to identify one such factor.

In order to reveal the factor, we first had to recruit subjects with the same systemic immunologic changes. In our study, all subjects in Groups 1 and 2 proved to be atopic. Data of serum total IgE levels and eosinophil counts showed that the parameters in Groups 1 and 2 were significantly higher than those of the controls. Studies have indicated that peripheral blood eosinophil levels may reflect the severity (15), hyper-responsiveness of the airway (16), and airway mucosal inflammation (17). The peripheral IgE level may be a marker itself of airway inflammation in patients with allergic disease (18). In our study subjects (Groups 1 and 2), serum IgE levels and eosinophil counts did not differ between the two groups (Table 1). From these data, we noted that subjects in Groups 1 and 2 had the same 'systemic immunologic changes' and the same levels of 'airway inflammation.'

Factors other than ACE polymorphism that may contribute to the development of different phenotypes must be excluded. In patients with allergic rhinitis, mouth breathing (19), post-nasal drip (20), and the nasal bronchial reflex (21) may contribute to the development of bronchial hyper-responsiveness. In subjects with only asthma, the confounding factors from rhinitis mentioned above are not seen. So we compared the occurrence of asthma in subjects with allergic rhinitis rather than comparing those with only asthma. Thus, the factors of asthma formation from rhinitis should be found in both groups rather than in only one group; and the bias would decrease. Other confounding factors such as a higher peripheral eosinophil count (17) and the presence of indoor allergens (22) may increase the frequency of asthma formation. In our study, the peripheral eosinophil counts did not differ (Table 1), allergens in Taiwan are almost all indoor allergens (23), and the result of allergen-specific IgE levels (Table 2) showed that allergen specificity were almost the same between the two groups. Thus, the effects of these two confounding factors could be reduced.

With the same systemic immunologic changes, the same allergen sensitivities in both groups, and excluding other factors that may have contributed to the development of asthma phenotype formation, we then compared the association of

Table 3. Genotype frequencies of the ACE gene polymorphism

	Group 1 AR (n = 106)	Group 2 AR + AS (n = 105)	Group 3 Control (n = 102)
Genotypes			
II	52 (49.1%)	48 (45.7%)	56 (54.9%)
ID	48 (45.3%)	40 (38.1%)	42 (41.2%)
DD	6 (5.7%)	17 (16.2%)	4 (3.9%)
DD	6 (5.7%)	17 (16.2%)	
Non-DD (ID + II)	100 (94.3%)	88 (83.8%)	
p-value (Group 1 vs. 2)*	0.018		
OR with 95% CI*	3.257 (1.222–8.680)		

Values are given as number (%).

Compare frequencies of genotypes between Groups 1 and 2, Groups 1 and 3, Groups 2 and 3, chi-square test $p = 0.046$, 0.674 and 0.013, respectively. AR, allergic rhinitis; AS, asthma; ACE, angiotensin-converting enzyme.

*Compare frequencies of DD genotype between Groups 1 and 2, Logistic regression analysis was used to adjust the potential confounder's gender and age.

the ACE genotypes with different phenotypes. In our study results (Table 3), we found that in Group 2 (allergic rhinitis with asthma), the frequency of the DD genotype was higher than in Group 1 (allergic rhinitis without asthma). Those with DD genotype were 3.257 times more likely to have asthma than those with ID and II genotypes.

Angiotensin-converting enzyme plays a role in converting angiotensin I to angiotensin II (24). Angiotensin II is a potent airway smooth muscle contractile agonist (6). ACE polymorphism DD genotype was associated with the highest serum ACE levels (25). We hypothesized that in children with allergic rhinitis, the frequency of DD genotype might be higher in subjects with asthma than those without asthma. This might be explained that in subjects with DD genotype, their serum ACE levels were higher, then the possibility of bronchoconstriction increase. Our study supported our hypothesis. It indicated that in children with allergic rhinitis, ACE polymorphism might play a role in the development of asthma. However, this polymorphism certainly was not the major factor, because there still is a large proportions of children with asthma where DD genotype were not found.

Some studies did not find the association of ACE polymorphism and asthma (26, 27). The discrepancy may be explained by the different ethnic backgrounds, different study design and environmental difference. Other studies indicated that the DD genotype is associated with asthma formation (7–10). But in the study by Benessiano et al. (7), Urhan et al. (9), and Gao et al. (10), they did not compare asthma with allergic rhinitis at the same time. Holla et al. (8) found that ACE gene polymorphism was associated with atopic disease, but they did not determine whether or not the polymorphism was associated with different phenotypes. A study by Kim et al. (28) indicated that ACE gene polymorphism was not associated with allergic rhinitis, but they did not compare it with asthma at the same time. No studies have directly investigated the role of ACE gene polymorphism in the formation of different phenotypes. We found the association of ACE polymorphism and asthma in Taiwanese children. We first investigated the association of ACE gene polymorphism in different phenotypes and then investigated the role of ACE gene polymorphism in asthma phenotype formation in children with allergic rhinitis.

There were some drawbacks of our study. First, the age of asthma development was younger than 8 yr in our previous study (29), younger than our study age. So that only five

subjects might develop asthma phenotype after the onset of allergic rhinitis in our study. Most children develop asthma at the same time of the onset of allergic rhinitis. Recruiting subjects with asthma after the onset of allergic rhinitis is necessary. Second, our study duration was only 2 yr. The observed association might be related to the early onset of asthma symptoms. Longer duration of study is necessary to study the association of late onset asthma symptoms.

Why some atopic subjects with allergic rhinitis develop asthma and others do not is still not fully understood. In our study, we found that the genetic factor, DD genotype of ACE genetic polymorphism, is associated with higher risk of asthma development in children with allergic rhinitis. It is not sufficient to say that the polymorphism is the cause of asthma phenotypes in allergic rhinitis children, and there must be other factors that play a role in the development of asthma phenotype. Future study is necessary.

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