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한국인에서 고친화성 감마글로불린-E 수용체 β사슬의 E237G 유전자다형과 기도과민성과의 연관성

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Distinct association between the E237G polymorphism of the high-affinity IgE receptor beta chain and airway hyperresponsivness in the korean general population

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目 次

表目次

圖目次

要 約

한국인에서 고친화성 감마글로불린-E 수용체 β사슬의 E237G 유전자다형과 기도과민성과의 연관성

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지도교수 : 이 승 일

의학과

조선대학교 대학원

연구배경 및 목적 : FcεR1는 호염기구와 비반세포에 존재하는 IgE에 대한 고친화성 IgE 수용체로 아토피와 천식의 발생에 관여한다. 천식 환자에서 FcεR1-β의 단일염기변이(exon7의 E237G)와 천식의 중간표현형인 호염기구 히스타민 유리능과의 연관성이 보고된 바 있다. 본 연구에서는 대규모의 역학조사를 바탕으로 하여 한국인에서 FcεR1-β의 유전자다형의 빈도를 확인하고, 아토피 및 기관지천식의 표현형인 기도과민성과의 연관성을 알아보고자 하였다.

연구계획 및 내용 : 제주도에 거주하는 소아 및 청소년 2,863명을 대상으로 최근 1년 동안의 천명의 유무, 비염 및 아토피피부염의 증상에 대한 설문조사를 시행하였고, 피부단자시험과 메타콜린 기관지유발시험을 통해 아토피 및 기도과민성 유무를 평가하였다. 말초혈액에서 DNA를 추출하여 FcεR1-β(E237G)의 유전형을 자동화된 단일염기변이 분석법을 이용하여 확인한 후, multiple logistic regression model을 이용하여 각종 표현형과의 연관성 분석을 시행하였다.

결과 : FcεR1-β과 관련된 축삭(axon)에서 의미있는 단일유전자다형은 E237G가 발견되었으며 이중 E237 유전자가 열성으로 빈도는 21.7%였다. E237 유전

자를 가진군에서 G237 유전자를 가진군보다 기도과민성 발현율이 낮게 관찰되었다[OR (95% CI) = 0.41 (0.19-0.89) p=0.01]. E237 유전자형군은 성별[남성: OR (95% CI) = 0.5 (0.4-0.63)], 알레르기 질환의 가족력[OR (95% CI) = 1.65 (1.28-2.13)]과 관련이 있었으나 혈청 감마글로불린-E 농도와는 연관이 없었다. 흥미있는 것은 현성 천명음과 기관지천식은 E237 유전다형군에서 좀 더 흔하게 관찰되었다[천명음; OR (95% CI) = 1.7 (1.03-2.82)], 기관지천식; OR (95% CI) = 2.45 (1.03-5.83)].

결론 : 한국인에게서 고친화성 감마글로불린-E 베타사슬(FcεRI-β)의 E237G 단일유전자다형은 기도과민성과 관련이 있으며 이 유전자형의 분석은 한국인에서 천식의 감수성을 예측할 수 있는 유전적 지표로서 중요하다.

INTRODUCTION

Asthma is an inflammatory airway disease associated with intermittent respiratory symptoms, such as wheezing and nocturnal cough, airway hyperresponsiveness (AHR) and reversible airflow obstruction, and is phenotypically heterogeneous.[1-3] Genetic studies in asthma families have suggested a genetic component.[4] Atopy, a genetic predisposition to asthma and other allergic diseases, is characterized by enhanced IgE responses to common allergens.[5] Airway hyperresponsiveness is believed to be essential in the development of asthma.[6] The Th2 cytokines, especially IL-13, are increased in the asthmatic airways and enhance AHR in normal unsensitized animals.[7] In terms of phenotypes asthma has complex phenotypes, and its intermediate phenotypes such as atopy and AHR play a key role in the pathogenesis of asthma.[8]

The high-affinity IgE receptor is expressed on the surface of mast cells and basophils. It is a transmembrane protein with one α, one β and two ɣ subunits.[9] The cDNA sequences for the receptor have already been determined.[10-12] Cross linking by multivalent allergens results in the aggregation of the bound IgE and α chain complexes at the cell surface, triggering cell activation, and subsequent internalization through coated pits.[13] The cytoplasmic domains of these subunits such as β and ɣ chains are important for intracellular signaling and the deduced amino acid sequences show the expected immunoreceptor tyrosine-based activation motifs (ITAMs).[14-17]The ɣ subunit is highly conserved between

species but more variation is seen with the β subunit.[12]

Previous linkage studies have suggested that atopy, but not AHR, is linked to the β chain of the high affinity IgE receptor (FcεRI-β) on chromosome 11q13.[1,18-20] The coding regions of FcεRI-β contain some non-synonymous single nucleotide polymorphisms.[21,22] Of those SNPs, an adenine to guanine substitution changes amino acid residue 237 from glutamic acid to glycine (E237G), in the cytoplasmic tail of the protein. The E237G is predicted to introduce a hydrophobicity change within the C-terminus of FcεRI-β. It is adjacent to the ITAM of FcεRI-β, and may affect the intracellular signaling capacity of FcεRI.[23]

Mediator release from inflammatory cells may be influenced by genetic factors.[24] Previous family study revealed that IgE receptor-mediated histamine release from basophils linked to the genetic marker of chromosome 11q13, and that the E237G polymorphism of FcεRI-β is significantly associated with histamine release from basophils in asthmatic children. However, there is no population-based case-control study to evaluate the genetic association between the E237G and asthma, atopy and AHR. Here I hypothesized that the E237G is significantly associated with asthma and its intermediate phenotypes, such as IgE responses to common allergens and AHR in the general population.

METHODS

STUDY SUBJECT

  Two thousand one hundred eighteen subjects aged from 10 to 18 years living in a rural area of Jeju island in Korea were recruited, and 1,033 male and 1,085 female. All the subjects responded to a questionnaire, and 9.5% and 12.4% of the questionnaire responders had experienced recurrent wheeze and nocturnal cough during the last 12 months, respectively. Skin prick testing to locally common aeroallergens and methacholine bronchial provocation testing were performed, and 36.9% and 25.5% of the subjects tested showed positive responses, respectively. They gave their blood for serum total IgE levels and genotyping, and their parents gave written informed consent for the study. This study protocol was approved by the Ethics Committee of Seoul National University Hospital.

CLINCAL PHENOTYPING

  A questionnaire developed by International Study of Asthma and Allergic disease in Children (ISAAC) was translated into Korean following the guideline laid down by ISAAC as previously described.[25] The questions on asthma symptoms concentrated on recurrent wheeze and nocturnal cough without respiratory tract infections during the previous 12 months. The questions on risk factors consisted of family history of allergic diseases, and history of passive smoking and vaccination, including measles, M. tuberculosis, and hepatitis B virus.

None of the subjects had received oral or inhaled bronchodilators for five days preceding methacholine bronchial provocation testing. Subjects with upper respiratory tract infections during the last two weeks were excluded from the methacholine challenge. A total of 2,055 subjects underwent the methacholine challenge as previously described.[25] Methacholine bronchial provocation testing was performed as previously described.[25] Methacholine AHR was expressed as PC20, and regarded as positive in this respect if the PC20 was lower than 16 mg/mL.

To evaluate IgE responses to common allergens, serum total IgE levels and skin prick testing to locally common aeroallergens were performed. Total serum IgE levels were determined in 2,058 subjects by using an ELISA.[4] Subjects who received oral antihistamines during the last five days preceding the skin prick testing or had dermographism were excluded from the skin prick testing. A total of 2,047 subjects underwent the testing. The skin testing with 11 locally common aeroallergens (Allergopharma, Germany) was performed.[3] Atopy was defined by positive skin prick test responses to one or more allergens, and skin index by sum of allergens showing positive skin test response. Classical asthma was defined when a subject with current wheeze on the questionnaire showed a positive AHR, and cough variant asthma when a non-wheezing subject with nocturnal cough on the questionnaire showed a positive AHR.

GENOTYPING

SNP scoring of the E237G was performed by SNP-IT^{TM} assays using SNP stream 25K^{TM} System which is customized to perform fully automated SNP genotyping of DNA samples in 384 well plates with a colorimetric readout (Orchid Biosciences, New Jersey, USA). Briefly, a single-base extension (SBE) primer was designed to be approximately 25 bp in length on one side of the SNP site. Automated liquid handling robotics were used to set up 5 μL PCR reactions in 384 well microtitre plates. Each PCR reaction contained: 10.0 ng of DNA, 1x PCR Buffer, 0.125 units of *AmpliTaq Gold* DNA polymerase (ABI, USA), 3.0 mM MgCl$_2$, 0.25 mM of each dNTP, and 0.5 pmol of each primer. Reactions were incubated at 95℃ for 10 minutes, then cycled 35 times at 95℃ for 30 s, 50, 60℃ for 1 min, and 72℃ for 1 min followed by 72℃ for 5 min. The amplified PCR products were digested with T7 exonuclease (0.45 U/μL) at room temperature for 30 min. The 5' phosphthioates on one of the PCR primer protected one strand of the PCR product from T7 exonuclease digestion, resulting in the generation of a single-stranded PCR product. The single-stranded PCR product was hybridized to a 384 well plate that contain covalently attached SNP-IT^{TM} primer extension primer designed to hybridize immediately adjacent to the SNP. After hybridization, the SNP-IT^{TM} primer was extended for a single base with Klenow fragment of DNA polymerase I and mixture of appropriate labeled terminating nucleotides which were labeled with either FITC or biotin and complementary to the SNP. The identity of the incorporated nucleotide was determined with serial colorimetric reactions with anti-FITC-AP

(alkaline phosphatase) and streptavidin-HRP (horse radish peroxidase) using p-nitrophenyl phosphate (PNPP) and tetramethylbenzidine (TMB) as a substrate respectively. The results of yellow and/or blue color developments for each sample were analyzed with ELISA reader and the final genotype calls were automatically assigned with QCReview^{TM} program (Orchid Biosciences). Automated genotype calls were corroborated by visual inspection of the data.

STATISTICAL ANALYSIS

To ensure that primary conclusions were robust to modeling assumptions, genotype was examined as a three level factored variable (for example, AA, AB, and BB; A is an allele of major frequency and B is an allele of minor frequency). To examine whether one of the homozygous genotypes has an effect that differs from a common effect of the heterozygous genotype and the other homozygote (AA vs. AB + BB or BB vs. AB + AA, dominant or recessive models, respectively). I performed 2x2 contingency table and multiple logistic regression modeling to adjust confounding variables such as age, sex, family history of allergic diseases, and history of passive smoking and vaccination. The log-transformed serum total IgE levels exhibited right skew and thus the statistical significance was evaluated by non-parametric methods. Hardy-Weinberg equilibrium was tested by χ^2 tests. All statistical analysis was performed using software SAS (version 8.1, Cary, NC, USA). A *P* value of .05 or less was regarded as significant.

RESULTS

GENE SCORING (Figure 1)

 The nonsynonimous SNP, resulting in Glu to Gly substitution, scoring of FcεR1-β genethat Korean have a mutant heterozygote (E237G) allele(20.01%) and a mutant homozyge (G237G) allele (1.51%).

RELATIONSHIP BETWEEN RISK FACTORS OTHER THAN GENETIC VARIATIONS AND THE EXPRESSION OF ATOPY AND AHR.

 In terms of relationship between the positive rate of atopy and risk factors including sex, family history of allergic diseases, and passive smoking history as shown in Table 1, the present study shows that atopy rate was significantly higher among boys than among girls [41.0% vs. 33.0%, $P < 0.001$, RR (95% CI) = 1.41 (1.18-1.69)]. In addition, atopy rate was found to be higher among subjects with a family history of allergic diseases than among those without [43.8% vs. 34.9%, $P < 0.001$, RR (95% CI) = 1.45(1.16-1.82)]. However, the positive rate of atopy was similar in subjects with and without passive smoking.

 Table 2 shows the effects of various risk factors including atopy on the expression of methacholine AHR. Interestingly, the present study shows that the positive rate of methacholine AHR was significantly lower among boys than among girls [18.5% vs. 32.2%, $P < 0.001$, RR (95% CI) = 0.48 (0.39-0.59)]. In addition, AHR positivity was significantly higher among subjects with a family history of allergic disease than among those without [34.5% vs. 18.3%, $P < 0.001$, RR (95% CI) = 1.73 (1.37-2.20)],

and among atopic subjects than among non-atopic ones [31.4% vs. 22.0%, P < 0.001, RR (95% CI) = 1.62 (1.32-1.98)]. However, AHR positivity was not related with passive smoking history (25.5% vs. 25.9%).

NO ASSOCIATION OF THE E237G WITH IG-E RESPONSES TO ALLERGENS (Table 3)

 IgE responses to allergens are important risk factors for asthma and have genetic components, and thus I hypothesized that the E237G is associated with IgE responses to common allergens, such as atopy, skin index, and total IgE in the general population. This study shows that the E237G was not significantly associated with atopy [OR (95% CI) = 1.11 (0.9-1.38)], skin index, and serum total IgE levels, although other risk factors, such as gender and family history of allergic diseases are significantly associated with it [male gender: OR (95% CI) = 1.46 (1.27-1.67); OR (95% CI) = 1.52 (1.28-1.79), respectively].

ASSOCIATION OF THE E237G WITH METHACHOLINE AHR (Table 4)

  I hypothesized that the E237G is associated with methacholine AHR in the general population. The E 237 allele was less frequent among subjects with enhanced AHR [OR (95% CI) = 0.41 (0.19-0.89)], which is also associated with age [OR (95% CI) = 1.12 (1.04-1.2)], gender [male: OR (95% CI) = 0.5 (0.4-0.63)], and family history of allergic diseases [OR (95% CI) = 1.65 (1.28-2.13)]. According to atopic status, the E 237 allele was less frequent among atopic subjects with enhanced AHR versus without it [OR (95% CI) = 0.29 (0.1-0.84)], which is also associated with

age [OR (95% CI) = 1.13 (1.04-1.23)], gender [male: OR (95% CI) = 0.48 (0.37-0.62)], and family history of allergic diseases [OR (95% CI) = 1.74 (1.31-2.31)]. However, in nonatopic subjects, the E 237 allele was not associated with enhanced AHR [OR (95% CI) = 0.83 (0.62-1.13)], although age, gender, and family history of allergic diseases was associated with it [OR (95% CI) = 1.18 (1.1-1.26) male gender: OR (95% CI) = 0.45 (0.36-0.56) OR (95% CI) = 1.78 (1.39-2.27), respectively].

ASSOCIATION OF THE E237G WITH CURRENT WHEEZE, BUT NOT WITH NOCTURNAL COUGH (Table 5)

I hypothesized that the E237G is associated with asthma symptoms, such as current wheeze and nocturnal cough in the general population. In contrast with the negative association of the E 237 allele with enhanced AHR, the E 237 allele was more common among subjects with current wheeze [OR (95% CI) = 1.7 (1.03-2.82)], which is also associated with family history of allergic diseases [OR (95% CI) = 2.42 (1.65-3.55)]. Interestingly, in subjects with enhanced AHR, the E 237 allele was also more common among subjects with current wheeze versus without it [OR (95% CI) = 3.2 (1.32-7.75)], which is also associated with family history of allergic diseases [OR (95% CI) = 3.09 (1.73-5.5)]. According to atopic status, in nonatopic subjects, the E 237 allele was more common among subjects with current wheeze versus without it [OR (95% CI) = 2.24 (1.05-4.77)], although, in atopic subjects, the E 237 allele was similar among subjects with current wheeze and without it [OR (95% CI) = 1.33 (0.67-2.63)].

As for the association of the E237G with nocturnal cough, the E237G was not associated with nocturnal cough [OR (95% CI) = 0.91 (0.67-1.24)], although other risk factors, such as age, gender, and family history of allergic diseases were significantly associated with it [OR (95% CI) = 1.08 (1.01-1.16)] male: OR (95% CI) = 0.61(0.49-0.75) OR (95% CI) = 2.65 (2.13-3.29), respectively]. Moreover, the E237G was not associated with nocturnal cough in both atopic and nonatopic subjects.

ASSOICATION OF THE E237G WITH CLASSICAL ASTHMA, BUT NOT WITH COUGH VARIANT ASTHMA (Table 6)

I hypothesized that the E237G is associated with asthma subtypes, such as classical asthma and cough variant asthma in the general population. As for classical asthma defined by current wheezing on the questionnaire plus enhanced AHR, the E 237 allele was more common among subjects with classical asthma [OR (95% CI) = 2.45 (1.03-5.83)], which is also associated with family history of allergic diseases [OR (95% CI) = 4.6 (2.68-7.88)]. According to atopic status, in nonatopic subjects, the E 237 allele was more common among subjects with classical asthma versus without it [OR (95% CI) = 2.23 (1.04-6.4)], although, in atopic subjects, the E 237 allele was similar among subjects with classical asthma and without it [OR (95% CI) = 1.75 (0.6-5.05)].

In terms of association between the E237G and cough variant asthma, the E237G was not associated with the prevalence of cough variant asthma in the general population [OR (95% CI) = 1.07 (0.57-2.0)], although other risk factors, such as age, gender, and family history of

allergic diseases were significantly associated with its prevalence [OR (95% CI) = 1.17 (1.03−1.33) male gender: OR (95% CI) = 0.3 (0.19−0.47) OR(95% CI) = 3.37 (2.26−5.02), respectively]. Moreover, the E237G was not associated with the prevalence of cough variant asthma according to atopic status.

DISCUSSION

Association studies using SNPs, which account for more than 90% of genetic variations, within candidate genes have the potential to identify genetic factors associated with complex traits, such as asthma and allergic diseases, and are generally more powerful than linkage-based approaches.[26] To the best of our knowledge, this study is the first large-scale population-based case-control study to evaluate the association between genetic variations of FcεRI-c (E237G) and asthma and its intermediate phenotypes.

The FcεRI on mast cells and basophils is a heterotetramer of polypeptide subunits which consists an IgE binding α-chain, a β-chain and a homodimer of ɣ-chains.[15] A number of studies have revealed that the β-chain, like the ɣ-chain, contains a 19-amino acid motif in its cytoplasmic tail, which is both necessary and sufficient to mediate signaling through receptors. However, the functional significance of E237G to FcεRI-β-mediated signaling is not yet known. Interestingly, E237G lies adjacent to the ITAM of FcεRI-β.[27] Two forms of ITAM appear in FcεRI, one in the β-chain, the other in the ɣ-chain. It has been proposed that they operate synergistically, and associate with specific protein tyrosine kinases, such as Lyn and Syk, which are capable of triggering cell activation via protein-tyrosine phosphorylation.[28] A Glu Gly substitution in the cytoplasmic tail (E237G) alters the hydrophilic nature of the C-terminus of the b-chain adjacent to ITAM by the substitution of a polar uncharged glycine for the larger negatively charged polar glutamic

acid residue. This change may alter the intracellular signaling capacity of FcεRI through the interaction of the protein tyrosine kinase Lyn with the ITAM of the β-chain. Our previous association study found that the E237G was significantly associated with FcεRI-mediated histamine release from basophils, suggesting that this nonsynonymous coding SNP in FceRI-β may result in a modulation of FcεRI signaling and then mediator release in response to receptor cross-linking.[29]

It is clear that Th2 immune responses to common environmental aeroallergens are the most important factor for the development of childhood asthma.[25] The findings that atopy defined by enhanced IgE responses to common allergens has familial predisposition from our previous family study,[4]and that it is significantly associated with family history of allergic diseases in the present study suggest that genetic components are important risk factor in the development of atopy.[4] Previous studies found a genetic linkage between markers in chromosome 11q13, where FcεRI-β gene is located, and atopy,[1,5,20,22] while other linkage studies including in Korean families found no evidence of the genetic linkage.[18,30,31] Surprisingly, there was no population-based case-control study to evaluate the association between the E237G and atopy. The present population-based case-control study shows that the E237G was not associated with IgE immune responses to common aeroallergens, suggesting that other genetic factors plays a key role in the Th2 immune responses to common allergens.

AHR is closely associated with asthma in schoolchildren, and is believed to be an important risk factor in the development of asthma

symptoms.[32] Mast cell infiltration of the airways is Th2 cytokine-dependent, and the difference between asthma and eosinophilic bronchitis (a condition characterized by recurrent cough, but not wheeze or AHR) may be infiltration of airway smooth muscle (ASM) by mast cells.[33] AHR, which include enhancing phenotypes, such as increased ASM hyperplasia and enhanced ASM contractility, and inhibitory phenotypes, such as subepithelial and lung parenchymal fibrosis, may all be important determinants of AHR expression.[34-36] Mediators from mast cells, such as tryptase and IL-13 induce the development of AHR in mice.[33, 37] Although the above findings suggest that mediators from mast cells play important roles in the development of AHR, there is no population-based study to demonstrate the association between genetic variations of FcεRI-β and AHR. The present study shows that the E 237 allele is less frequent among subjects with enhanced AHR. Interestingly, the association of the E237G with AHR is more prominent in atopic subjects, but not in nonatopic subjects. These findings provide the importance of FcεRI-β gene in the genetic predisposition to AHR in atopic subjects.

Variable airway constriction and then recurrent wheezing are characteristic clinical manifestations of asthma, but the underlying mechanisms for the development of wheezing are known to be distinct from the development of cough.[38] The mast cell is localized at the interface of the internal and external environment within the lung where it may respond to allergens and other nonspecific stimuli. The activation of mast cells leads to the release of mediators that contribute to the early

phase of airway constriction and the latephase of asthmatic inflammation.[33] These findings suggest that mast cell mediators via high-affinity IgE receptor-mediated signaling pathways play important roles in the development of variable airway constriction and the resultant recurrent wheezing. Indeed, the present study shows that the E237G is significantly associated with current wheeze, but not with nocturnal cough, in the general population, suggesting that genetic predisposition to these two phenotypes is distinct.

The present study also shows that the E237G was significantly associated with current wheeze both in subjects with enhanced AHR and in nonatopic subjects, but not in atopic subjects. These findings suggest that FcεRI-β partly determine the genetic predisposition to airway constriction and then wheezing mediated by mast cell activation, especially to exogenous or endogenous stimuli other than allergens. Interestingly, the present study also shows that E 237 allele was more common among subjects having enhanced AHR with current wheeze than without it. Meanwhile, this allele was less frequent among subjects with enhanced AHR than without it. These findings suggest that the development of AHR and clinical manifestations of asthma is distinct conditions in their genetic predisposition.

CONCLUSION

This is the first report demonstrating significant association and functional relevance of non-synonymous SNP (E237G) of FcεRI-β with AHR. It is known that IgE receptor-mediated histamine release from basophils is significantly higher in asthmatics than in non-asthmatic controls. Previous family study revealed that IgE receptor-mediated histamine release from basophils linked to the genetic marker of chromosome 11q13 where β-subunit of high affinity IgE FcεRI-β gene is located. The aim of this study was to evaluate the association between single nucleotide polymorphism(E237G) of the FcεRI-β gene and skin test responses to aeroallergens, and other intermediate phenotypes associated with asthma in the Korean population. The nonsynonimous SNP, resulting in Glu to Gly substitution, scoring of FcεR1-β genethat Korean have a mutant heterozygote (E237G) allele(20.01%) and a mutant homozyge (G237G) allele (1.51%). There is association between the atopy and the nonsynonimous SNP "E237G" of FcεRI-β gene, and association between the airways hyperresponsiveness.

In conclusion, this study provides new evidences that clinical manifestations of asthma are distinct processes with the development of airway hyperresponsiveness in terms of genetic predisposition. These findings indicate the importance of E237G allele as a genetic marker to predict asthma susceptibility in the Korean population.

REFERENCES

1. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet 1989; 1:1292-5.

2. Kim YK, Baek D, Koh YI, Cho SH, Choi IS, Min KU, et al. Outdoor air pollutants derived from industrial processes may be causally related to the development of asthma in children. Ann Allergy Asthma Immunol 2001; 86:456-60.

3. Kim YK, Chang YS, Lee MH, Hong SC, Bae JM, Jee YK, et al. Role of environmental exposure to spider mites in the sensitization and the clinical manifestation of asthma and rhinitis in children and adolescents living in rural and urban areas. Clin Exp Allergy 2002; 32:1305-9.

4. Kim YK, Cho SH, Koh YY, Son JW, Jee YK, Lee MH, et al. Skin reactivity to inhalant allergens, total serumIgE levels, and bronchial responsiveness to methacholine are increased in parents of nonatopic asthmatic children. J Allergy Clin Immunol 1999; 104:311-6.

5. Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, et al. Localisation of atopy andbeta subunit of high-affinity IgE receptor (Fc epsilon RI) on chromosome 11q. Lancet 1993; 341:332-4.

6. Townley RG, Horiba M. Airway hyperresponsiveness: a story of mice and men and cytokines. Clin Rev Allergy Immunol 2003;

24:85-110.

7.   Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998; 282:2258-61.

8.   Ahmadi KR, Goldstein DB. Multifactorial diseases: asthma genetics point the way. Curr Biol 2002; 12:R702-4.

9.   Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP. Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 1989; 337:187-9.

10.   Goitsuka R, Hayashi N, Nagase M, Sasaki N, Ra C, Tsujimoto H, et al. Molecular cloning of cDNAs encoding dog high-affinity IgE receptor alpha, beta, and gamma chains. Immunogenetics 1999; 49:580-2.

11.   Maekawa K, Imagawa N, Tanaka Y, Harada S. Determination of the sequence coding for the beta subunit of the human high-affinity IgE receptor. FEBS Lett 1992; 302:161-5.

12.   McAleese SM, Miller HR. Cloning and sequencing of the equine and ovine high-affinity IgE receptor beta-and gamma-chain cDNA. Immunogenetics 2003; 55:122-5.

13.   Bingham CO, 3rd, Austen KF. Mast-cell responses in the development of asthma. J Allergy Clin Immunol 2000; 105:S527-34.

14.   Kimura T, Sakamoto H, Appella E, Siraganian RP. The negative signaling molecule SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP) binds to the tyrosine-phosphorylated beta subunit of the high affinity IgE receptor. J Biol Chem 1997;

272:13991-6.

15.   Alber G, Miller L, Jelsema CL, Varin-Blank N, Metzger H. Structure-function relationships in the mast cell high affinity receptor for IgE. Role of the cytoplasmic domains and of the beta subunit. J Biol Chem 1991; 266:22613-20.

16.   Takahashi K, Nishiyama C, Hasegawa M, Akizawa Y, Ra C. Regulation of the human high affinity IgE receptor beta-chain gene expression via an intronic element. J Immunol 2003; 171:2478-84.

17.   Takahashi K, Matsumoto C, Ra C. FHL3 negatively regulates the human high affinity IgE receptor beta-chain gene expression by acting as a transcriptional co-repressor of MZF-1. Biochem J 2004; Pt.

18.   Martinati LC, Trabetti E, Casartelli A, Boner AL, Pignatti PF. Affected sib-pair and mutation analyses of the high affinity IgE receptor beta chain locus in Italian families with atopic asthmatic children. Am J Respir Crit Care Med 1996; 153:1682-5.

19.   Palmer LJ, Daniels SE, Rye PJ, Gibson NA, Tay GK, Cookson WO, et al. Linkage of chromosome 5q and 11q gene markers to asthma-associated quantitative traits in Australian children. Am J Respir Crit Care Med 1998; 158:1825-30.

20.   Shirakawa T, Hashimoto T, Furuyama J, Takeshita T, Morimoto K. Linkage between severe atopy and chromosome 11q13 in Japanese families. Clin Genet 1994; 46:228-32.

21.   Zhang X, Zhang W, Qiu D, Sandford A, Tan WC. The E237G polymorphism of the high-affinity IgE receptor beta chain and

asthma. Ann Allergy Asthma Immunol 2004; 93:499-503.

22.　　Shirakawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, et al. Association between atopy and variants of the beta subunit of the high-affinity immunoglobulin E receptor. Nat Genet 1994; 7:125-9.

23.　　Hill MR, Cookson WO. A new variant of the beta subunit of the high-affinity receptor for immunoglobulin E (Fc epsilon RI-beta E237G): associations with measures of atopy and bronchial hyper-responsiveness. Hum Mol Genet 1996; 5:959-62.

24.　　Marone G, Poto S, Celestino D, Bonini S. Human basophil releasability. III. Genetic control of human basophil releasability. J Immunol 1986; 137:3588-92.

25.　　Kim YK, Park HS, Kim HY, Jee YK, Son JW, Bae JM, et al. Citrus red mite (Panonychus citri) may be an important allergen in the development of asthma among exposed children. Clin Exp Allergy 2001; 31:582-9.

26.　　Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996; 273:1516-7.

27.　　Reth M. Antigen receptor tail clue. Nature 1989; 338:383-4.

28.　　Jouvin MH, Adamczewski M, Numerof R, Letourneur O, Valle A, Kinet JP. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. J Biol Chem 1994; 269:5918-25.

29.　　Kim YK, Oh SY, Oh HB, Chun SY, Cho SH, Koh YY, et al. Coding single nucleotide polymorphism in the high-affinity immunoglobulin

E receptor b chain (FcepsilonRI-beta) gene is associated with immunoglobulin E receptor-mediated histamine release from basophils. Clin Exp Allergy 2002; 32:751-5.

30.   Xu J, Postma DS, Howard TD, Koppelman GH, Zheng SL, Stine OC, et al. Major genes regulating total serum immunoglobulin E levels in families with asthma. Am J Hum Genet 2000; 67:1163-73.

31.   Cho SH, Son JW, Koh YY, Min KU, Kim YY, Kim YK. Linkage between bronchial responsiveness to methacholine and gene markers of IL-4 cytokine gene cluster and T-cell receptor alpha/delta gene complex in Korean nuclear families. Clin Exp Allergy 2001; 31:103-9.

32.   Clarke JR, Salmon B, Silverman M. Bronchial responsiveness in the neonatal period as a risk factor for wheezing in infancy. Am J Respir Crit Care Med 1995; 151:1434-40.

33.   Bradding P. The role of the mast cell in asthma: a reassessment. Curr Opin Allergy Clin Immunol 2003; 3:45-50.

34.   Hargreave FE, Dolovich J, O'Byrne PM, Ramsdale EH, Daniel EE. The origin of airway hyperresponsiveness. J Allergy Clin Immunol 1986; 78:825-32.

35.   Pare PD. Airway hyperresponsiveness in asthma: geometry is not everything! Am J Respir Crit Care Med 2003; 168:913-4.

36.   Boulet LP, Turcotte H, Laviolette M, Naud F, Bernier MC, Martel S, et al. Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Influence of inhaled corticosteroids. Am J Respir Crit

Care Med 2000; 162:1308-13.

37.   Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999; 103:779-88.

38.   Choudry NB, Fuller RW, Anderson N, Karlsson JA. Separation of cough and reflex bronchoconstriction by inhaled local anaesthetics. Eur Respir J 1990; 3:579-83.

Table 1. Relationship between risk factors and the prevalence of atopy

		Subjects		P *	RR (95% CI)[†]
		Atopy[‡]	Non-atopy		
Age(mean ± SD)		14.58±1.42	14.61±1.43		
Sex	Boy	409 (41.0%)	589	<0.001	1.41(1.18-1.69)
	Girl	346 (33.0%)	703		
Family history[§]	+	172 (43.8%)	221	<0.001	1.45(1.16-1.82)
	−	565 (34.9%)	1054		
Smoking[¶]	+	509 (35.9%)	910	0.38	0.91(0.75-1.11)
	−	226 (38.0%)	368		

* Chi-square test

[†] Multiple logistic regression analysis after adjusting for other confounding factors

[‡] Positive skin test responses to one or more common allergens

[§]Family history of allergic disease by a questionnaire

[¶] Passive smoking history by a questionnaire

Table 2. Relationship between risk factors and airway hyperresponsiveness

		Subjects		P *	RR (95% CI)[†]
		AHR(+)[‡]	AHR(−)		
Age(mean ± SD)		14.61±1.48	14.53±1.41		
Sex	Boy	185 (18.5%)	815	<0.001	0.48(0.39−0.59)
	Girl	340 (32.2%)	715		
Family history[§]	+	137 (34.5%)	260	<0.001	1.73(1.37−2.20)
	−	278 (18.3%)	1243		
Smoking[¶]	+	363 (25.5%)	1063	0.88	0.98(0.79−1.22)
	−	154 (25.9%)	441		
Atopy[¥]	+	234 (31.4%)	512	<0.001	1.62(1.32−1.98)
	−	282 (22.0%)	997		

* Chi−square test

[†] Multiple logistic regression analysis after adjusting for other confounding factors

[‡] PC20−methacholine < 16 mg/ml

[§] Family history of allergic disease by a questionnaire

[¶] Passive smoking history by a questionnaire

[¥] Positive skin test responses to one or more common allergens

Table 3. The genotype frequencies of E237G of FceRI-β according to IgE responses to common aeroallergens in the general population

Genotypes		E237G			P *	OR (95% CI)[†]
Phenotypes		EE	EG	GG		
Atopy[‡]	+	568 (80.8%)	120 (17.1%)	15 (2.1%)	0.23	1.11 (0.9-1.38)
	−	936 (77.4%)	259 (21.4%)	15 (1.2%)		
Skin index[§]		2.50 ± 1.43	2.71 ± 1.71	2.20 ± 1.32	0.3	
log(total IgE)[¶]		1.86 ± 0.78	1.83 ± 0.80	1.87 ± 0.76	0.27	

* Chi-square test

[†] Multiple logistic regression analysis after adjusting for confounders, such as, age, sex, family history of allergic diseases, and history of passive smoking and vaccination.

[‡] Positive skin test responses to one or more common aeroallergens, expressed by No (%).

[§] Number of allergens showingpositive skin test responses, expressed by mean ± SD.

[¶] Mean ± SD.

Table 4. The genotype frequencies of E237G of FceRI-β according to methacholine airway hyperresponsiveness in the general population

Genotypes		E237G			P *	OR (95% CI)[†]
Phenotypes		EE	EG	GG		
AHR[‡]	+	372 (76.2%)	103 (21.1%)	13 (2.7%)	0.01	0.41 (0.19-0.89)
	−	1116 (79.4%)	274 (19.5%)	16 (1.1%)		
Atopic AHR[§]	+	169 (77.5%)	40 (18.4%)	9 (4.1%)	0.01	0.29 (0.1-0.84)
	−	383 (82.2%)	77 (16.5%)	6 (1.3%)		
Nonatopic AHR[¶]	+	196 (74.8%)	62 (23.7%)	4 (1.5%)	0.26	0.83 (0.62-1.13)
	−	717 (77.9%)	193 (21.0%)	10 (1.1%)		

* Chi-square test

[†] Multiple logistic regression analysis after adjusting for confounders, such as, age, sex, family history of allergic diseases, and history of passive smoking and vaccination.

[‡] PC_{20}-methacholine < 16 mg/ml, expressed by No (%) and analyzed by dominant model of genotypes (EE + EG vs. GG).

[§] PC_{20}-methacholine < 16 mg/ml in atopic subjects, expressed by No (%) and analyzed by dominant model of genotypes (EE + EG vs. GG).

[¶] PC_{20}-methacholine < 16 mg/ml in non-atopic subjects, expressed by No (%).

Table 5. The genotype frequencies of E237G of FceRI-β according to asthma symptoms in the general population

Genotypes		E237G			*P* *	OR (95% CI)[†]
Phenotypes		EE	EG	GG		
Current wheeze[‡]	+	118 (86.1%)	18 (13.2%)	1 (0.7%)	0.02	1.7 (1.03–2.82)
	−	1371 (78.0%)	359 (20.4%)	28 (1.6%)		
Symptomatic AHR[§]	+	56 (90.3%)	6 (9.7%)	0	0.004	3.2 (1.32–7.75)
	−	304 (74.0%)	94 (22.9%)	13 (3.1%)		
Atopic wheeze[¶]	+	56 (86.2%)	8 (12.3%)	1 (1.5%)	0.23	1.33 (0.67–2.63)
	−	489 (80.0%)	108 (17.7%)	14 (2.3%)		
Nonatopic wheeze[¶]	+	59 (88.0%)	8 (12.0%)	0	0.03	2.24 (1.05–4.77)
	−	830(76.6%)	239 (22.1%)	14 (1.3%)		
Nocturnal cough[¥]	+	175 (76.4%)	50 (21.8%)	4 (1.8%)	0.42	0.91 (0.67–1.24)
	−	1268 (78.8%)	317 (19.7%)	25 (1.5%)		
Atopic cough[£]	+				0.06	0.64 (0.4–1.02)
	−					
Nonatopic cough[£]	+				0.85	1.11 (0.73–1.65)
	−					

* Chi-square test

[†] Multiple logistic regression analysis after adjusting for confounders, such as, age, sex, family history of allergic diseases, and history of passive smoking and vaccination.

[‡] Wheezing during the previous 12 months on the questionnaire, expressed by No (%) and analyzed by recessive model of genotypes (EE

vs. EG + EE).

§ Current wheeze in subjects with enhanced methacholine AHR, expressed by No (%) and analyzed by recessive model of genotypes (EE vs. EG + EE).

¶ Current wheeze in subjects with and without positive skin test responses to common allergens, expressed by No (%).

¥ Recurrent nocturnal cough without viral respiratory infections on the questionnaire, expressed by No (%).

£ Nocturnal cough in subjects with and without positive skin test responses to common allergens, expressed by No (%).

Table 6. The genotype frequencies of E237G of FceRI-β according to asthma subtypes in the general population

Genotypes		E237G			P *	OR (95% CI)[†]
Phenotypes		EE	EG	GG		
Classical asthma[‡]	+	56 (90.3%)	6 (9.7%)	0	0.03	2.45 (1.03-5.83)
	−	1021 (79.1%)	254 (19.7%)	15 (1.2%)		
Atopic asthma[§]	+	34 (89.5%)	4 (10.5%)	0	0.21	1.75 (0.6-5.05)
	−	350 (85.2%)	71 (16.7%)	5 (1.1%)		
Nonatopic asthma[§]	+	22 (91.7%)	2 (8.3%)	0	0.03	2.23 (1.04-6.4)
	−	384 (82.8%)	75 (16.1%)	5 (1.1%)		
CVA[¶]	+	48 (81.4%)	10 (16.9%)	1 (1.7%)	0.94	1.07 (0.57-2.0)
	−	883 (80.3%)	204 (18.6%)	12 (1.1%)		
Atopic CVA[✶]	+				0.3	0.68 (0.26-1.72)
	−					
Nonatopic CVA[✶]	+				0.47	1.39 (0.57-3.36)
	−					

* Chi-square test

[†] Multiple logistic regression analysis after adjusting for confounders, such as, age, sex, family history of allergic diseases, and history of passive smoking and vaccination.

[‡] Current wheeze on the questionnaire in subjects with enhanced airway hyperresponsiveness, expressed by No (%)and analyzed by recessive model of genotypes (EE vs. EG + GG).

[§] Classical asthma in subjects with and without positive skin test

responses to common allergens, expressed by No (%) and analyzed by recessive model of genotypes (EE vs. EG + GG).

¶ Cough variant asthma defined by recurrent nocturnal cough in subjects with enhanced airway hyperresponsiveness, expressed by No (%).

¥ Cough variant asthma in subjects with and without positive skin test responses to common allergens, expressed by No (%).
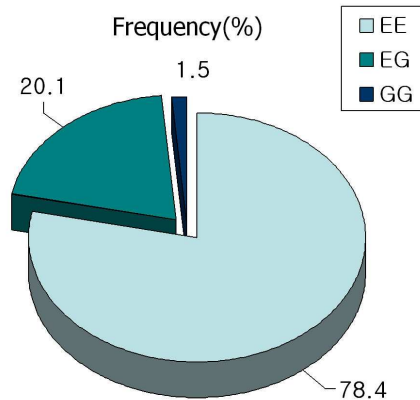
Figure 1. SNP scoring of FcεR1-β subunit gene

FcεR1-β gene SNP (E237G)

: Nucleotide   Adenine → Guanine

: Aminoacid
    Glutaminc acid(E) → Glycine(G)

The SNP scoring of FcεR1-β subunit gene show that Glutamic acid is most prominent amino acid in 237 codon and Korean have a mutant heterozygote EG genotype about 20% and a mutant homozygote GG genotype (1.5%).

APPENDIX

Abbreviations used:

AHR: airway hyperresponsiveness(기도과민성)

ASM: airway smooth muscle(기도평활근)

CI: confidence Interval(신뢰수준)

E237G: glutamic acid glycine at codon 237 of FceRI-β

FceRI-β: high-affinity IgE receptor beta chain(고친화성 감마글로불린-E 수용체)

ITAM: immunoreceptor tyrosine activation motif

OR: odd ratio(교차비)

RR: relative risk(상대위험도)

SNP: single nucleotide polymorphism(단일유전자다형)