Title page

Title: Staphylococcal enterotoxin sensitization and late-onset asthma in adults

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Take home message

Staphylococcal enterotoxin sensitization was highly prevalent and independently associated with late-onset asthma.

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Abstract

Recent studies suggest that *Staphylococcus aureus* enterotoxin sensitization is a risk factor for asthma. However, there is still limited epidemiologic evidence to support the associations in adult asthma.

The present analyses were performed using the baseline dataset of Korean adult population surveys, consisting of 1,080 adults (mean age = 60.2 years) recruited from two rural community areas. Questionnaires, methacholine challenge tests, allergen skin tests and stool exams (*Clonorchis sinensis eggs*) were performed for defining clinical phenotypes. Sera were analyzed for total IgE and enterotoxin specific IgE using ImmunoCAP.

Staphylococcal enterotoxin sensitization (≥ 0.35 kU/L) had a prevalence of 27.0%. Risk factors were identified as male sex, current smoking, advanced age, and atopy. Current asthma had a prevalence of 5.5%, and was mostly late in onset (93.8%). Using multivariate logistic regression, late-onset asthma was independently associated with high enterotoxin specific IgE levels. In multivariate linear regressions, Staphylococcal enterotoxin specific IgE level was identified as the major determinant for total IgE level.

These findings suggest the clinical significance of Staphylococcal enterotoxin sensitization in late-onset adult asthma.

Word count: 174

Key words: asthma; bacteria; epidemiology of asthma

Introduction

Asthma is now considered as a heterogeneous disorder [1] . Particularly, adult-onset asthma may have more complex pathogenic mechanisms other than the conventional Th2-mediated model which is largely mediated by atopy [1, 2].

Staphylococcus aureus is a human commensal microorganism that is responsible for various invasive infectious diseases [3]. However, the superantigenic properties of Staphylococcal enterotoxins (SE) [4] have been also associated with allergic diseases in the skin [5] and upper airways [6].

Recently, immune responses driven by SE have been linked to adult asthma [6, 7]. This relationship was supported by case-control studies [7-11] and meta-analyses [12, 13] of associations between SE-specific IgE (SE-sIgE) levels and asthma. More recently, a GA^2LEN survey was conducted to investigate the association for the first time in the general adult population [14]. However, further evidence is needed to prove the roles of SE sensitization in adult asthma.

The present analysis aimed to investigate the epidemiologic features and the clinical significance of SE sensitization, including risk factors and relationships with asthma and allergic parameters, using epidemiologic surveys of Korean adult community populations.

Methods

Study population

Cross-sectional surveys were previously conducted on adults living in two regions in Korea in 2007 [15]. The two study regions are endemic for clonorchiasis and in Gyeongnam Province: Shinan-meon in Sancheong and Buk-meon in Changwon. Both regions are mainly agricultural areas, but Sancheong is more rural than Changwon. The surveys were conducted by collaboration between the Clinical Research Center for Chronic Obstructive Airway Disease of Seoul National University Hospital and Seoul National University Bundang Hospital and the National Cancer Center of Korea. The study protocol was reviewed and approved by the institutional review board.

Study participants were recruited in collaboration with Public Health Centers in the study regions. Briefly, a leaflet and information materials detailing the study purpose and protocols were distributed to residents by the head of each village. The residents were contacted directly or by telephone calls for enrollment. In total, 1,116 subjects who were at least 30 years of age recruited voluntarily from a target population of 17,494 residents (616 subjects from 5,526 Sancheong residents and 500 subjects from 11,968 Changwon residents). All participants provided written informed consent. Our final analysis included 1,080 subjects (96.8%) who had serum available for IgE measurements.

Questionnaires

Interviews were performed by trained researchers who were certified to conduct epidemiological surveys. The structured questionnaires included demographic parameters, medical history and allergic disease history. Medical histories included items on 40 common

chronic illnesses. The presence of allergic diseases was assessed using questionnaires on asthma and rhinitis, as described previously [16].

Phenotype definitions

Current asthma was defined positive if the subjects had current wheeze ("Have you had a wheezing or whistling in the chest during the last 12 months?") and methacholine airway hyperresponsiveness (AHR), or had used asthma medication within the last 12 months (defined as recent asthma treatment). Past asthma was defined by prior asthma diagnosis in history but no recent symptoms, negative AHR, and no asthma treatment within the last 12 months. Asthma was further classified by a participant-recalled asthma onset of 40 years as 'early-onset' or 'late-onset'. Rhinitis was defined by the question "Have you had a problem with sneezing or a runny or blocked nose without a cold in the last 12 months?"

Methacholine challenge tests

Methacholine challenge tests were performed using a modification of Chai's method to measure AHR. Lung functions were measured by a portable spirometer (Micro Spirometer, Micro Medical, Kent, UK). Subjects with respiratory infections within the previous two weeks were excluded to avoid false-positive AHR. Subjects with a baseline forced expiratory volume 1 s (FEV1) of lower than either 1,200 mL or 50% of the predicted value were also excluded because of the presence of chronic obstructive pulmonary disease. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA) was used to deliver aerosols generated by a nebulizer (DeVilbliss, Carlsbad, CA, USA). Subjects were instructed to inhale five inspiratory capacity breaths of increasing methacholine concentration (1.25, 2.5, 6.25, 12.5, and 25 mg/mL) until the FEV1 decreased to < 80% of the baseline value or until the highest concentration was reached. Triplicate FEV1 measurements were

recorded at 90 or 180 s after each inhalation, and the highest value was selected for analysis. Methacholine AHR was scored as positive if the concentration of methacholine provoking a 20% decrease in FEV1 was < 16 mg/mL.

Allergen skin prick test

Skin testing was performed using a panel of 10 common inhalant allergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog epithelia, *Blatella germanica*, fungus mixture, tree pollen mixture 1 (alder, hazel, popular, elm, and willow), tree pollen mixture 2 (birch, beech, oak, and plane tree), grass pollen mixture (velvet grass, orchard grass, rye grass, timothy grass, Kentucky blue grass, and meadow grass), mugwort, and ragweed. Positive (1 mg/mL histamine) and negative (0.9% sodium chloride) controls were also tested. The skin response was defined positive if the allergen wheal was larger than or equal in size to the histamine wheal and the diameter of the allergen wheal was at least 3 mm. Atopy was determined when the subjects had a positive skin response to any of the tested allergens.

Serum total IgE and specific IgE measurements for staphylococcal enterotoxin

Serum samples were assayed for total IgE and SE-sIgE levels using the ImmunoCAP 250 (Thermo Fischer, Uppsala, Sweden) according to the manufacturer's instructions. SE-sIgE was measured using a Staphylococcal enterotoxin mix (SEA, SEC and TSST-1; Thermo Fischer, Uppsala, Sweden), as previously described [7].

Other assessments

Height and weight were measured to the nearest 0.1 cm or 0.1 kg in barefoot individuals wearing light clothing. Body mass index (BMI) was calculated by dividing weight by height squared. Stool samples were analyzed for *Clonorchis sinensis* infection using the Kato-Katz

method, and the intensity of infection was described as eggs per gram of feces (EPG) as previously described [15].

Statistical analyses

Descriptive data were calculated as the mean ± standard deviation, median [interquartile range (IQR)], or percentages. Data were compared by a t-test, Mann-Whitney test, one-way ANOVA or chi-squared test. Correlations between continuous variables were evaluated using Spearman bivariate tests. Logistic regression tests were performed to identify risk factors for SE sensitization or asthma. Factors for adjustments were selected when $P < 0.10$ in univariate logistic regression tests or if they were traditional risk factors. Multiple correspondence analyses were used to determine the relationships between identified risk factors and SE sensitization. For the asthma logistic regression test, the control group was defined as subjects that did not have current or past asthma. Linear regression models were used to identify determinants for total IgE levels. All the statistical analyses were performed using the Stata 12.0 software package (Stata Corporation, College Station, TX, USA).

Results

Baseline characteristics

In total, 1,080 subjects were included in the analysis (Table 1). The population had a mean age of 60.2 ± 11.5 yrs, was predominantly female (62.8%) and had an average BMI for Korean adults (mean 23.6 ± 3.2 kg/m^2), with no differences between the Sancheong and Changwon areas. The prevalence of atopy and current asthma was 12.0% and 5.5%, respectively. Most of current asthma (93.8%) was late-onset. The prevalence of SE sensitization was 27.0% when defined by the ≥ 0.35 kU/L cutoff (or 55.7% by the ≥ 0.10 kU/L cutoff) without significant difference between areas. The prevalence of SE sensitization, atopy and asthma was presented in Figure 1 by age group.

Risk factors for Staphylococcal enterotoxin (SE) sensitization

First, we explored risk factors for SE sensitization. In univariate chi-squared tests (Supplementary Table E1), the relevant factors were identified as male sex, advanced age, atopy, current smoking, current alcohol use, diabetes mellitus and *Clonorchis* infection. Using multivariate logistic regression tests (Table 2), independent associations with SE sensitization were tested. Male sex, advanced age (≥ 61 years), atopy and current smoking were identified as independent risk factors for sensitization. In Spearman tests, serum SE-sIgE levels showed a positive correlation with pack-year smoking history (r = 0.247, P < 0.001). Diabetes mellitus was the only comorbid condition that was related to SE sensitization in univariate tests but only marginally related in multivariate tests (P = 0.057). Obesity (defined as BMI ≥ 27.5 kg/m^2) tended to increase the risk of SE sensitization (P = 0.085). Multiple correspondence analyses were performed to confirm the relationships between SE

sensitization and six relevant factors that were identified in multivariate logistic regression tests ($P < 0.10$). SE sensitization was closely correlated with male sex and smoking (Figure 2).

Associations between SE sensitization and atopy

The relationship between atopy and SE sensitization was further investigated. Statistical significance was found for most tested allergens including house dust mites, cockroaches, grass pollen, and tree pollens. The number of allergen sensitizations was also significantly correlated with SE sensitization (likelihood-ratio $P = 0.002$; Supplementary Table E2). After inclusion of SE sensitization as an additional independent variable, risk factors for atopy were investigated in multivariate logistic regression tests. To study dose relationships, SE-sIgE levels were categorized into tertile-based groups (T1: 0–0.06; T2: 0.07–0.26; T3: 0.27–50.68 kU/L). High SE-sIgE showed a significant association with atopy [T3 vs. T1; odds ratio (OR) 2.14, 95% confidence interval (CI) 1.26–3.65, $P = 0.005$] (Supplementary Table E3).

Associations between SE sensitization and asthma

Levels of SE-sIgE were compared according to the age of asthma onset and current clinical disease activity. Late-onset current asthma had significantly higher rates of SE sensitization compared to never asthma or other asthma phenotypes (Table 3); of interest, the late-onset current asthma was mostly non-atopic. To investigate independent associations between SE sensitization and late-onset current asthma, multivariate logistic regression tests were performed, with adjustments for SE sensitization risk factors, traditional risk factors (atopy, obesity and rhinitis), and demographic factors (residence area, age and sex). Late-onset current asthma was significantly associated with SE sensitization (OR 2.41, 95% CI 1.28–4.55, $P = 0.006$). Using tertile-based SE-sIgE categories, the dose relationship confirmed the significant association with high SE-sIgE levels (Table 4).

Correlations between total IgE and SE-sIgE levels

To test potential confounding effects between total IgE and SE-sIgE, their correlations were evaluated in linear regression models. In univariate linear regressions, relevant factors for total IgE levels were SE-sIgE ($P < 0.001$), sex ($P < 0.001$), smoking status ($P < 0.001$), *Clonorchis* infection ($P < 0.001$), atopy ($P = 0.058$), and alcohol use ($P = 0.084$). Multivariate linear regression analyses demonstrated that SE-sIgE was a stronger determining factor (Table 5) than other parameters including male sex, current smoking, alcohol use, *Clonorchis* infection (or infection intensity defined by EPG) and atopy. The strong correlation between SE-sIgE and total IgE (Spearman's $r = 0.758$, $P < 0.001$) was also presented in a scatter plot, as log-transformed (Figure 3).

Discussion

The present analysis demonstrated that SE sensitization was highly prevalent and independently associated with late-onset current asthma among community-based older adult populations in Korea. SE sensitization had a strong positive relationship with advanced age (≥ 61 years), male sex, current smoking and atopy. In addition, we found that SE-sIgE levels were the major determinants for total IgE.

SE-sIgE is an emerging marker for asthma [7, 9]. However, there is limited evidence of its association with asthma in general population samples. In children/adolescents, two studies have been published on the association between SE sensitization and asthma [510 five-year-old children in the UK [17] and 1,380 fourteen-year-old children in Australia [18]]. In adults, the GA^{2}LEN survey has recently reported the significant links with asthma in a large-scale European general population (n=2,908, mean age 48.9 years) [14]. In this regard, the present study is addition to the knowledge in that this is the first community population-based report in Asian adults. Considering the homogeneous results across various European centers in GA^{2}LEN, the present study supports the notion that the asthma-SE sIgE association is universally significant across continents and various age groups.

Moreover, we here for the first time report that SE sensitization is significantly related to late-onset adult asthma. As shown in Figure 1, the prevalence of asthma and SE sensitization increased with aging, whereas atopy decreased. A low atopy rate (5.3%) among late-onset older adult asthmatics indicates less impact of atopy in those subjects, compared to a younger population of adults [7]. As asthma is a heterogeneous disorder [1] and SE sensitization rate ranges from 38 to 76% of adult asthmatics [12], it is still uncertain that how SE is exactly involved in the asthma pathophysiology. However, we have shown before that specifically

severe non-atopic adult asthma was highly associated with SE-IgE [7], which supports the present findings.

In this regard, the relationship between older age and SE sensitization is of note. As skin from older individuals has a reduced barrier function or an increased epidermal permeability to exogenous antigens [19], we presume that the risk of sensitization increases with aging. Compared with previous population-based studies, the SE sensitization rate was significantly higher among our control subjects (mean age 60.2 years, 26.1%) than among younger controls in the previous two studies [mean age 5 years, 7.5% in UK children [17] and mean age 14 years, 17.9% in Australian controls [18]]. Although these data are not directly comparable because of substantial demographic differences, age could have an effect on SE sensitization, such that 'older age' may be a risk factor for SE sensitization and subsequently for late-onset adult asthma.

Smoking is another risk factor for SE sensitization, which may have significant clinical implications. In our various statistical models and also in the GA^{2}LEN analyses [14], smoking status was consistently found to have positive and close correlations with SE-sIgE levels. As *S. aureus* is inhaled in indoor dust and frequently colonizes the upper airways [3], we postulate that epithelial damage caused by smoke exposure increases the risk of staphylococcal enterotoxin penetration into subepithelial layers, thereby leading to SE-mediated stimulation of immune cells and sensitization.

Interestingly, we found no independent effects of smoking on asthma when co-adjusted with SE sensitization which had stronger effects, despite that smoking is known as a risk factor for adult asthma [2]. Further studies are necessary to draw firm conclusions; however, it may be speculated that SE sensitization and smoking have common pathways in the asthma

pathogenesis, and that prior evidence for smoking on asthma could have been co-mediated by SE sensitization effects, at least in part.

Our findings on the association with atopy confirm previous results from children/adolescent population studies [17, 18] and extend this knowledge into older adults. As it was a cross-sectional analysis, we could not determine causal relationships between atopy and SE sensitization. However, on the basis of age-related prevalence patterns, we speculate that atopy could be a predisposing factor for SE sensitization. Atopic subjects may be more prone to *S. aureus* colonization and sensitization, as M2 macrophages, which are more frequent in the Th2 milieu, have reduced phagocytotic activity, which leads to increased risk of bacterial survival [20]. Conversely, there is also experimental evidence that nasal Staphylococcal enterotoxin B (SEB) administration enhances allergen sensitization in mice [21], and in *in vitro* studies using human dendritic cells (DC), SEB caused DCs to drive the Th2 polarization of naïve T cells [22].

The identification of SE-sIgE as a major determinant of total IgE level is another interesting finding. Total IgE, which is a traditional indicator of allergy, may have significant associations with demographic or environmental factors [23, 24], genetic factors [25], atopy [24] or parasitic infection [26]. We performed a comprehensive analysis of IgE in areas with high rates of endemic *Clonorchis* infection to identify the major determinants of total IgE levels. Interestingly, SE-sIgE levels were the most significant determinant, most likely because SE is a superantigen that promotes polyclonal IgE production [4]. These findings suggest that the interpretation of total IgE levels in previous studies should be reevaluated. Moreover, the potential confounding effects of total IgE need to be considered when interpreting multivariate analyses. Clinically, adult asthmatics frequently have increased serum total IgE but no evidence of atopy or parasitic infection; this effect could possibly be explained by SE-

sIgE [7]. We suggest that SE-sIgE level should be included as a significant biomarker in epidemiological or clinical evaluations for adult allergic disorders.

There is overwhelming evidence in animal studies and human mucosal models that staphylococcal enterotoxins break tolerance [21] and induce airway inflammation and IgE synthesis [27, 28]; however, there is no direct evidence for *S. aureus* enterotoxin pathogenesis in human airway disease. Although *S. aureus* is known to reside in the upper airways and skin [3], its presence in the lower airways has been contested. However, recent advances in metagenomics have challenged the 'old dogma' by demonstrating that the lower respiratory tracts of asthma patients are not sterile but rather have a high burden of commensal bacteria [29, 30]. Therefore, further studies are needed to directly demonstrate the role of *S. aureus* in the pathogenesis of asthma.

The present study has several limitations. By using a cross-sectional survey, we could not determine causal relationships between parameters. Because the study participants were not randomly recruited but were volunteers from communities, selection biases may have existed. Recall bias may also be present, as outcomes like symptoms or medical history was based on participants' recall. The results could not be generalized because the survey was not conducted nationwide. In addition, we could not draw any conclusions on early-onset asthma, due to a small number of early-onset current asthma cases. However, the selection of well-defined local areas with endemic *Clonorchis* is a strength of the study. The demographic features of the present survey were different from previous studies [14, 17, 18]. Thus, the present findings are new addition to previous knowledge.

In conclusion, we demonstrated that Staphylococcal sensitization is highly prevalent and significantly associated with late-onset current asthma among older adult community populations. Our identification of risk factors for SE sensitization may be useful in

understanding the pathophysiology of SE-mediated allergic disorders. In addition, the strong correlation between SE-sIgE and total IgE levels is further addition to its clinical significance.

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Figure Legends

Figure 1. Age-related prevalence of current asthma, atopy and Staphylococcal enterotoxin sensitization among study population.

Figure 2. Multiple correspondence analysis map representing the relationships between Staphylococcal enterotoxin sensitization and six relevant factors that were identified in multivariate logistic regression tests

Figure 3. Correlation between log-transformed total IgE and Staphylococcal enterotoxin specific IgE (SE sIgE) levels. The scatter plot is shown with the 95% confidence interval (CI) and the regression line.

Table 1. Baseline characteristics of study population according to residence areas

Parameters	Sancheong (n=605)	Changwon (n=475)	*P* value
Age (yrs)	59.9 ± 12.1	60.6 ± 10.8	0.306
Female sex (%)	64.0	61.3	0.362
BMI (kg/m^2)	23.5 ± 3.2	23.8 ± 3.2	0.130
Smoking (%)			
Never smoker	64.4	65.0	0.435
Ex-smoker	17.3	19.3	
Current smoker	18.3	15.7	
Alcohol (%)			
Never drinker	50.5	54.0	0.080
Ex-drinker	8.9	5.3	
Current drinker	40.6	40.7	
Diabetes mellitus (%)	8.4	9.3	0.631
Rhinitis* (%)	16.9	16.1	0.706
Current asthma† (%)	5.8	6.1	0.954
Atopy‡ (%)	12.3	11.4	0.649
Clonorchis infection§ (%)	33.8	14.6	< 0.001
FVC%pred	92.4 ± 15.9	94.6 ± 17.2	0.037
FEV1%pred	108.3 ± 20.7	108.6 ± 20.6	0.891
FEV1/FVC%	84.7 ± 8.4	82.9 ± 9.4	0.002
SE-sIgE			
Levels (kU/L)	Median 0.14 (IQR 0.05–0.41)	Median 0.11 (IQR 0.04–0.37)	0.372
≥ 0.35 kU/L (%)	27.6	26.3	0.636
Total IgE (kU/L)	129.1 (IQR 43.4–358.2)	98.6 (IQR 35.9–307.3)	0.781

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; SE-sIgE, Staphylococcal enterotoxin specific IgE

*Rhinitis was defined by the question “Have you had a problem with sneezing, or a runny, or blocked nose without a cold in the last 12 months?”.

†Current asthma was defined as positive if the subjects had current wheeze (Have you had a wheezing or whistling in the chest during the last 12 months?) and methacholine airway hyperresponsiveness, or had used asthma medication within the last 12 months.

‡Atopy was defined as positive if the subjects had a positive skin response to any of tested 10 inhalant allergens.

§Clonorchis infection was defined by stool sample analyses for the presence of eggs.

P values were determined by t tests, Mann-Whitney tests, or chi-squared tests.

Table 2. Multivariate logistic regression for Staphylococcal enterotoxin sensitization

SE sensitization (≥ 0.35 kU/L) as dependent variable (292 cases vs. 788 controls)	Adjusted OR (95% CI)*	*P* value
Residence area		
Sancheong	Reference	
Changwon	1.02 (0.74–1.39)	0.922
Age group		
≤ 40 yrs	Reference	
41–50 yrs	1.77 (0.78–4.01)	0.173
51–60 yrs	2.17 (0.99–4.75)	0.053
61–70 yrs	2.74 (1.27–5.90)	0.010
> 70 yrs	2.95 (1.33–6.57)	0.008
Sex		
Female	Reference	
Male	1.93 (1.21–3.07)	0.006
BMI (kg/m^2)		
< 23	Reference	
23.0–24.9	0.85 (0.58–1.25)	0.414
25.0–27.4	1.19 (0.79–1.79)	0.418
≥ 27.5	1.58 (0.94–2.65)	0.085
Smoking		
Never smoker	Reference	
Ex-smoker	1.36 (0.82–2.25)	0.232
Current smoker	3.26 (2.03–5.24)	< 0.001
Alcohol		
Never drinker	Reference	
Ex-drinker	0.92 (0.50–1.71)	0.800
Current drinker	1.13 (0.78–1.64)	0.516
Diabetes mellitus		
No	Reference	
Yes	1.61 (0.99–2.64)	0.057
Atopy		
No	Reference	
Yes	2.28 (1.45–3.59)	< 0.001
Clonorchis infection		
No	Reference	
Yes	1.16 (0.83–1.68)	0.366

Abbreviations: SE, Staphylococcal enterotoxin; OR, odds ratio; CI, confidence interval; BMI, body mass index

Please see Table 1 for definitions.

P values were determined by multivariate logistic regression tests.

*Adjusted for residence area, age group, sex, BMI category, smoking status, alcohol status, atopy, *Clonorchis* infection, and diabetes mellitus.

Table 3. Phenotype comparisons by asthma onset and current symptom activity

	Never asthma[*] (n=992)	Past asthma[†] (n=28)	Early-onset current asthma[‡] (n=4)	Late-onset current asthma[§] (n=56)	*P* values
Age (yrs)	59.7 ± 11.6	63.6 ± 10.3	61.3 ± 6.6	66.7 ± 7.8	< 0.001[ll]
Asthma onset (yrs)		46.8 ± 20.0	30.3 ± 6.3	57.2 ± 12.8	0.003[ll]
Female sex (%)	62.7	75.0	75.0	57.6	0.437[¶]
BMI (kg/m^2)	23.6 ± 3.1	22.8 ± 3.0	25.4 ± 2.5	24.0 ± 4.2	0.229[ll]
Smoking (%)					
Never smoker	65.0	67.9	50.0	58.6	0.893[¶]
Ex-smoker	17.9	21.4	25.0	20.7	
Current smoker	17.1	10.7	25.0	20.7	
Rhinitis (%)	15.7	28.0	0	26.3	0.056[¶]
Atopy (%)	12.5	11.1	0	5.3	0.356[¶]
SE-sIgE (kU/L)	Median 0.12 (IQR 0.04–0.37)	Median 0.09 (IQR 0.03–0.15)	Median 0.12 (IQR 0.05–0.23)	Median 0.32 (IQR 0.17–0.91)	0.006[ll]
SE-sIgE ≥ 0.35 kU/L (%)	26.1	14.3	0.0	48.2	< 0.001[¶]

Abbreviations: BMI, body mass index; SE, Staphylococcal enterotoxin; IQR, inter-quartile ranges

See Table 1 for definition of current asthma, rhinitis and atopy.

[*]Never asthma was defined by no asthma diagnosis in history and no current asthma.

[†]Past asthma was defined by prior asthma diagnosis history but no current asthma.

[‡]Early-onset current asthma was defined by a participant-recalled asthma history starting before 40 years old, and also having current asthma.

[§]Late-onset current asthma was defined by a participant-recalled asthma history starting after 40 years old, and also having current asthma.

[ll]*P* values were determined by one-way ANOVA.

¶*P* values were determined by chi-squared tests.

Table 4. Multivariate logistic regression tests for late-onset current asthma

	Adjusted OR (95% CI)*	*P* value
Residence area		
Sancheong	Reference	
Changwon	1.07 (0.58–1.95)	0.836
Age	1.09 (1.05–1.13)	< 0.001
Sex		
Female	Reference	
Male	0.85 (0.36–.03)	0.719
BMI (kg/m^2)		
< 23	Reference	
23.0 – 24.9	0.98 (0.44–2.18)	0.954
25.0 – 27.4	1.05 (0.45–2.42)	0.918
≥ 27.5	2.94 (1.25–6.91)	0.013
Smoking		
Never	Reference	
Ex-	1.32 (0.49–3.56)	0.589
Current	1.96 (0.71–5.35)	0.192
Atopy		
No	Reference	
Yes	0.28 (0.06–1.22)	0.090
Rhinitis		
No	Reference	
Yes	2.17 (1.04–4.50)	0.038
SE-sIgE levels		
Lowest tertile (–0.06 kU/L)	Reference	
Mid-tertile (0.07–0.26 kU/L)	2.93 (1.10–7.82)	0.031
Highest tertile (0.27–50.68 kU/L)	5.32 (2.07–13.6)	0.001

Abbreviations: SE, Staphylococcal enterotoxin; OR, odds ratio; CI, confidence interval; BMI, body mass index

Please see Tables 1 and 3 for definitions.

P values were determined by multivariate logistic regression tests.

*Adjusted for residence area, age, sex, BMI category, smoking status, atopy, and rhinitis

Table 5. Multivariate linear regression for total IgE levels (kU/L)

	P values*	β	Std. Err.	t
SE-sIgE (kU/L)	< 0.001	144.77	12.25	11.82
Male sex	0.001	313.47	90.91	3.45
Smoking status				
Ex-smoking	0.712	36.78	99.62	0.37
Current smoking	0.016	233.58	97.00	2.41
Alcohol status				
Ex-drinker	0.472	-85.11	118.27	-0.72
Current drinker	0.032	142.95	66.40	2.15
Atopy	0.639	41.18	87.67	0.47
Clonorchis infection	0.043	131.78	65.07	2.03

Abbreviations: SE, Staphylococcal enterotoxin

Please see Table 1 for definitions.

**P* values were determined by multivariate linear regression tests with adjustment for SE sIgE, sex, smoking status, alcohol status, atopy, and *Clonorchis* infection.