

Staphylococcal enterotoxin sensitization in a community-based population: a potential role in adult-onset asthma

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Summary

Background Recent studies suggest that *Staphylococcus aureus* enterotoxin sensitization is a risk factor for asthma. However, there is a paucity of epidemiologic evidence on adult-onset asthma in community-based populations.

Objective We sought to evaluate the epidemiology and the clinical significance of staphylococcal enterotoxin sensitization in community-based adult populations.

Methods The present analyses were performed using the baseline data set of Korean adult population surveys, consisting of 1080 adults (mean age = 60.2 years) recruited from an urban and a rural community. Questionnaires, methacholine challenge tests, and allergen skin tests were performed for defining clinical phenotypes. Sera were analysed for total IgE and enterotoxin-specific IgE using ImmunoCAP.

Results Staphylococcal enterotoxin sensitization (≥ 0.35 kU/L) had a prevalence of 27.0%. Risk factors were identified as male sex, current smoking, advanced age (≥ 61 years), and inhalant allergen sensitization. Current asthma was mostly adult onset (≥ 18 years old) and showed independent associations with high enterotoxin-specific IgE levels in multivariate logistic regression tests. In multivariate linear regressions, staphylococcal enterotoxin-specific IgE level was identified as the major determinant factor for total IgE level. **Conclusions and Clinical Relevance** Staphylococcal enterotoxin sensitization was independently associated with adult-onset asthma in adult community populations. Strong correlations between the enterotoxin-specific IgE and total IgE levels support the clinical significance. The present findings warrant further studies for the precise roles of staphylococcal enterotoxin sensitization in the asthma pathogenesis.

Keywords asthma, epidemiology, immunoglobulin E, *Staphylococcus aureus*

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Introduction

Asthma is now considered as a heterogeneous disorder [1]. Particularly, adult-onset asthma is regarded as having more heterogeneous mechanisms and phenotypes than childhood-onset asthma, which has been largely associated with genetic factors or inhalant allergen sensitization [1, 2]. However, there is a paucity of evidence about the epidemiology and risk factors for adult-onset asthma.

Staphylococcus aureus is a human commensal micro-organism that is often 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-onset asthma [6, 7]. This relationship was supported by case-control studies [7–13]. More recently, a GA²LEN survey was conducted to investigate the association for the first time in the general adult population [14]. However, further evidence is needed to clarify the roles of SE sensitization in adult-onset 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.

Material and 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 Changwon is more urbanized than Sancheong. 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 of the National Cancer Center, Korea (IRB reference NCCNCS-07-080).

Study participants were recruited in collaboration with public health centres 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 enrolment. In total, 1116 subjects who were at least 30 years of age recruited voluntarily from a target population of 17 494 residents (616 subjects from 5526 Sancheong residents and 500 subjects from 11 968 Changwon residents). All participants provided written informed consent. Our final analysis included 1080 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]. 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?'

Definition of asthma

Current asthma was defined positive if the subjects had positive methacholine airway hyperresponsiveness (AHR) and current wheeze ('Have you had a wheezing or whistling in the chest during the last 12 months?').

Past asthma was defined by prior asthma diagnosis in history, but having no current wheeze or AHR. Asthma was further classified by a participant-recalled symptom onset of 18 years as 'childhood onset' or 'adult onset'.

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 1200 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 (DeVilbiss, 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, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog epithelia, *Blatella germanica*, fungus mixture, tree pollen mixture 1 (alder, hazel, poplar, 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 weal was larger than or equal in size to the histamine weal and the diameter of the allergen weal was at least 3 mm. Inhalant allergen sensitization 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 analysed 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 $\pm$ standard deviation, median [interquartile range (IQR)], or percentages. Data were compared by a *t*-test, Mann–Whitney test, one-way ANOVA test, or chi-squared test. Bivariate correlations were examined by Spearman's tests. Logistic regression tests were performed to identify independent risk factors for SE sensitization or asthma. In multivariate logistic regression tests for SE sensitization, confounders included variables with $P < 0.10$ in univariate tests (age, sex, smoking status, alcohol status, diabetes mellitus, inhalant allergen sensitization, and clonorchis infection) and also demographic factors (residence area and BMI). Multiple correspondence analyses were used to confirm the relationships between SE sensitization and the risk factors identified in multivariate logistic regression tests. For the asthma logistic regression test, the control group was defined as never having asthma; multivariate logistic regression tests were performed with adjustment for factors with $P < 0.10$ in univariate tests (age, BMI, smoking status, alcohol status, inhalant allergen sensitization, and rhinitis) and a demographic factor (sex). Linear regression models were used to identify determinants for total IgE levels. As total IgE and SE-sIgE levels were not normally distributed (Shapiro–Wilk W test: $W < 0.001$), they were transformed to logarithmic scales for linear regression tests. All the statistical analyses were performed using the Stata 12.0 software package (Stata Corporation, College Station, TX, USA).

Results

Baseline characteristics

In total, 1080 subjects were included in the analysis (Table 1). The population had a mean age of

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

Parameters	Sancheong (<i>n</i> = 605)	Changwon (<i>n</i> = 475)	<i>P</i> value
Age (years)	59.9 $\pm$ 12.1	60.6 $\pm$ 10.8	0.306
Female sex (%)	64.0	61.3	0.362
BMI (kg/m ²)	23.5 $\pm$ 3.2	23.8 $\pm$ 3.2	0.130
Smoking status (%)			
Never smoker	64.4	65.0	0.435
Ex-smoker	17.3	19.3	
Current smoker	18.3	15.7	
Alcohol status (%)			
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† (%)	5.6	6.1	0.836
Inhalant allergen sensitization‡ (%)	12.3	11.4	0.649
Clonorchis infection§ (%)	33.8	14.6	< 0.001
FVC%pred	92.4 $\pm$ 15.9	94.6 $\pm$ 17.2	0.037
FEV1%pred	108.3 $\pm$ 20.7	108.6 $\pm$ 20.6	0.891
FEV1/FVC%	84.7 $\pm$ 8.4	82.9 $\pm$ 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)	Median 129.1 (IQR 43.4–358.2)	Median 98.6 (IQR 35.9–307.3)	0.781

BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; SE-sIgE, staphylococcal enterotoxin-specific IgE; IQR, interquartile range.

*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.

‡Inhalant allergen sensitization 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.

60.2 $\pm$ 11.5 years, was predominantly female (62.8%), and had an average BMI for Korean adults (mean: 23.6 $\pm$ 3.2 kg/m²), with no differences between the Sancheong and Changwon areas. The prevalence of current asthma and past asthma was 5.8% and 2.6%, respectively. The prevalence of SE sensitization was 27.0% when defined by the ≥ 0.35 kU/L cut-off (or 55.7% by the ≥ 0.10 kU/L cut-off) without a significant difference between areas. The prevalence of inhalant allergen sensitization was 12.0%. The prevalence of

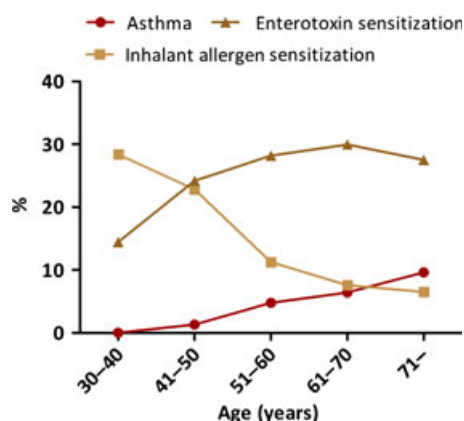


Fig. 1. Prevalence of current asthma, inhalant allergen sensitization and staphylococcal enterotoxin sensitization by age groups.

current asthma, inhalant allergen, and SE sensitization is shown in Fig. 1 by age groups.

Risk factors for staphylococcal enterotoxin sensitization

First, we explored the risk factors for SE sensitization. In univariate chi-squared tests (Table S1), the relevant factors were identified as male sex, advanced age, current smoking, current alcohol use, diabetes mellitus, inhalant allergen sensitization, and *Clonorchis* infection. Using multivariate logistic regression tests (Table 2), independent associations with SE sensitization were tested. Male sex, advanced age (≥ 61 years), current smoking, and inhalant allergen sensitization were identified as independent risk factors for SE sensitization. In Spearman's 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/m² [17]) 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 status (Fig. 2).

Associations between inhalant allergen and staphylococcal enterotoxin sensitization

The relationships between inhalant allergen and SE sensitizations were further investigated. Statistical significance was found for most tested allergens including house dust mites, cockroaches, grass pollen, and tree pollens. The number of allergen sen-

Table 2. Multivariate logistic regression for staphylococcal enterotoxin sensitization*

SE-positive sensitization (n = 292) vs. negative sensitization (n = 788)	Adjusted OR (95% CI) [†]	P-value
Residence area		
Sacheong	Reference	
Changwon	1.02 (0.74–1.39)	0.922
Age group (years)		
≤ 40	Reference	
41–50	1.77 (0.78–4.01)	0.173
51–60	2.17 (0.99–4.75)	0.053
61–70	2.74 (1.27–5.90)	0.010
> 70	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 status		
Never smoker	Reference	
Ex-smoker	1.36 (0.82–2.25)	0.232
Current smoker	3.26 (2.03–5.24)	< 0.001
Alcohol status		
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
Inhalant allergen sensitization		
No	Reference	
Yes	2.28 (1.45–3.59)	< 0.001
<i>Clonorchis</i> infection		
No	Reference	
Yes	1.16 (0.83–1.68)	0.366

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.

*Staphylococcal enterotoxin sensitization was defined by serum staphylococcal enterotoxin mix (SEA, SEC, and TSST-1)-specific IgE level ≥ 0.35 kU/L.

[†]Adjusted for residence area, age group, sex, BMI category, smoking status, alcohol status, diabetes mellitus, inhalant allergen sensitization, and *Clonorchis* infection.

sitizations was also significantly correlated with SE sensitization (likelihood ratio $P = 0.002$; Table S2). After inclusion of SE sensitization as an additional independent variable, risk factors for inhalant allergen sensitization were investigated in multivariate logistic regression tests. To study dose relationships, SE-sIgE levels were categorized into tertile-based

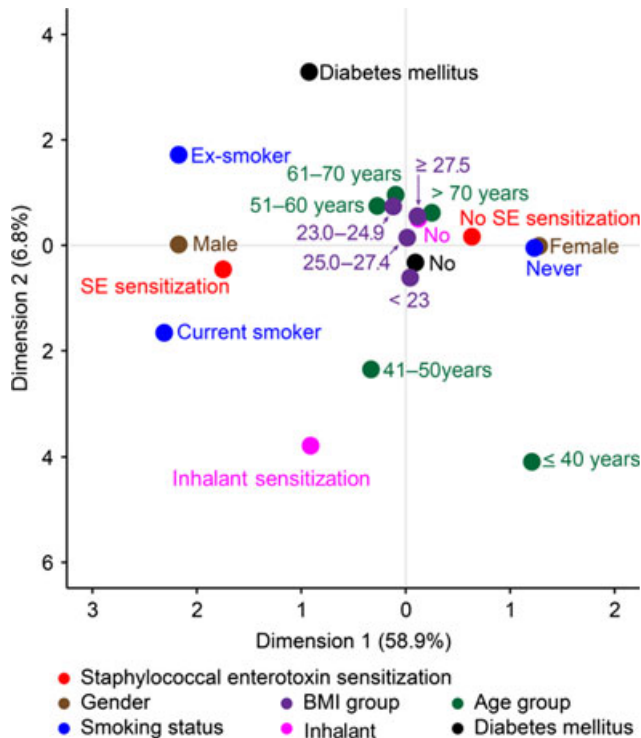


Fig. 2. Multiple correspondence analysis map representing the relationships between staphylococcal enterotoxin sensitization and six relevant factors that were identified in multivariate logistic regression tests

groups above 0.10 kU/L (T1: 0.10–0.22; T2: 0.22–0.61; and T3: 0.61–50.68 kU/L). High SE-sIgE showed a significant association with inhalant allergen sensitization [T3 vs. negative; odds ratio (OR): 3.13, 95% confidence interval (CI): 1.79–5.48, $P < 0.001$] (Table S3).

Correlations between total IgE and staphylococcal enterotoxin-specific IgE levels

The correlations between total IgE and SE-sIgE levels were evaluated in linear regression models. In univariate linear regressions, relevant factors for log-transformed total IgE levels were log-SE-sIgE ($P < 0.001$), age ($P = 0.022$), sex ($P < 0.001$), smoking status ($P < 0.001$), BMI ($P = 0.069$), alcohol use ($P < 0.001$), inhalant allergen sensitization ($P < 0.001$), and *Clonorchis* infection ($P < 0.001$). Multivariate linear regression analyses demonstrated that log-SE-sIgE was a stronger determining factor (Table 3) than other parameters including male sex, current smoking, alcohol use, *Clonorchis* infection (or infection intensity defined by EPG), and inhalant allergen sensitization. The correlation between SE-sIgE and total IgE levels ($r = 0.758$, $P < 0.001$) was also presented in a scatter plot, as log-transformed (Fig. 3).

Table 3. Multivariate linear regression tests for total IgE levels (kU/L)

For log-total IgE	<i>P</i> -values*	β	Std. Err.	<i>t</i>
Log-SE-sIgE (kU/L)	< 0.001	0.58	0.02	29.5
Male sex	< 0.001	0.49	0.10	5.05
Age	< 0.001	0.01	0.003	3.94
BMI	0.013	0.02	0.01	2.49
Smoking status				
Ex-smoker	0.457	−0.08	0.11	−0.74
Current smoker	0.904	−0.13	0.11	−0.12
Alcohol status				
Ex-drinker	0.374	0.11	0.13	0.89
Current drinker	< 0.001	0.32	0.07	4.37
Inhalant allergen sensitization	0.001	0.33	0.09	3.43
<i>Clonorchis</i> infection	0.008	0.19	0.07	2.67

SE-sIgE, staphylococcal enterotoxin-specific IgE; BMI, body mass index.

Please see Table 1 for definitions.

**P*-values were determined by multivariate linear regression tests with adjustment for log-transformed SE-sIgE, sex, age, BMI, smoking status, alcohol status, inhalant allergen sensitization, and *Clonorchis* infection.

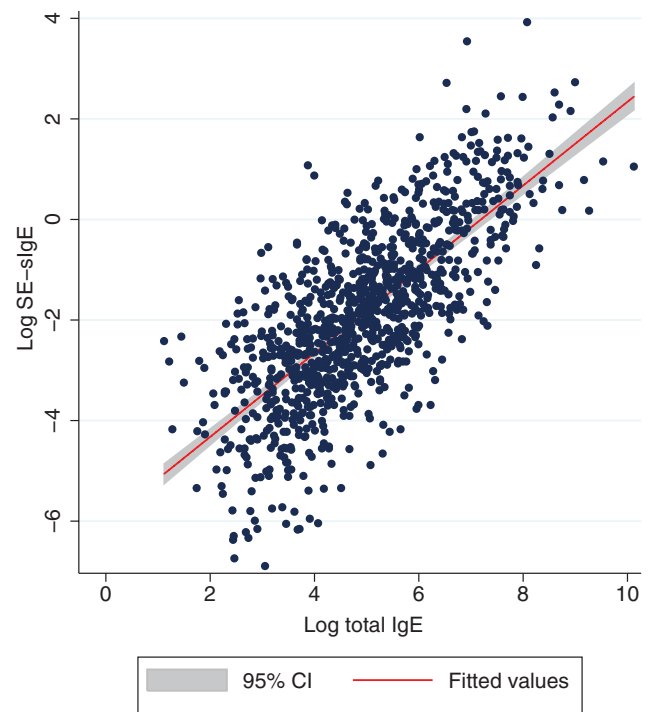


Fig. 3. Correlation between log-transformed total IgE and staphylococcal enterotoxin-specific IgE (SE-sIgE) levels. The scatter plot is shown with the 95% confidence interval (CI) and the regression line.

Associations between staphylococcal enterotoxin sensitization and asthma

Subjects with current asthma had a significantly higher rate of SE sensitization, or higher levels of SE-sIgE compared to those with never asthma or past asthma

(Table 4). To investigate independent associations between SE sensitization and current asthma, multivariate logistic regression tests were performed as described above. Current asthma was significantly associated with SE sensitization (OR: 2.52, 95% CI: 1.36–4.67, $P = 0.003$). Current smoking was related to current asthma in a univariate test (vs. never smoking; OR: 2.34, 95% CI: 1.08–5.04, $P = 0.020$); however, the relationship was not significant in multivariate models coadjusted with SE sensitization (OR: 1.47, 95% CI: 0.66–3.26, $P = 0.347$).

Of interest, current asthma was mostly adult onset (mean onset: 60.5 ± 10.3 years). Adult-onset current asthma ($n = 60$) had higher levels of SE-sIgE (median: 0.31, IQR: 0.17–0.91 kU/L) compared to childhood-onset current asthma ($n = 3$; median: 0.06, IQR: 0.03–0.27 kU/L), past asthma, or never asthma (Table 4). In multivariate logistic regression, adult-onset current asthma showed independent associations with SE sensitization (vs. never asthma; OR: 2.46, 95% CI: 1.34–4.54, $P = 0.004$). The positive associations between adult-onset current asthma and SE-sIgE levels were further supported by the dose relationships, using the tertile-based SE-sIgE categories (Table 5).

Discussion

The present analysis demonstrated the epidemiology and the significance of SE sensitization in the commu-

nity-based adult populations. Smoking history, male sex, older age (≥ 61 years), and inhalant allergen sensitization were risk factors for SE sensitization. SE-sIgE level was a strong determinant factor for total IgE level. The sensitization showed independent associations with adult-onset current asthma.

Recent evidence suggests that SE-sIgE is an emerging marker for asthma [7, 9]. However, there is a paucity of evidence in general populations. In children/adolescents, two studies have been published on the association between SE sensitization and asthma (510 five-year-old children in the United Kingdom [18] and 1380 fourteen-year-old children in Australia [19]). In adults, the GA²LEN survey has recently found the significant links with asthma in European general populations ($n = 2908$, mean age: 48.9 years) [14]. In this regard, the present study is addition to the knowledge, conducted for the first time in Asian general adult population. From the homogeneous results of various European centres in GA²LEN, this present study supports the notion that the asthma–SE association is universally significant across continents.

Moreover, we found that SE sensitization was significantly related to adult-onset current asthma in the community-based adult population. As the number of current asthma was very small among childhood-onset asthma, we could not draw conclusions on childhood-onset longstanding asthma. The low prevalence of childhood-onset asthma could have been attributed to

Table 4. Comparison by asthma onset and current activity

	Never asthma* ($n = 989$)	Past asthma† ($n = 28$)	Current asthma ($n = 63$)	<i>P</i> -values
Age (years)	59.7 ± 11.6	63.6 ± 10.3	$66.4 \pm 7.7^{\parallel}$	$< 0.001^{\S}$
Adult-onset asthma‡ (%)	–	50%	95.2%	0.003**
Female sex (%)	62.7	75.0	58.7	0.327**
BMI (kg/m ²)	23.6 ± 3.1	22.8 ± 3.0	24.1 ± 4.1	0.161 [§]
Smoking status (%)				
Never smoker	65.0	67.9	58.1	0.708**
Ex-smoker	17.9	21.4	21.0	
Current smoker	17.1	10.7	21.0	
Rhinitis (%)	15.7	28.0	24.6	0.058**
Inhalant allergen sensitization (%)	12.5	11.1	3.3	0.104**
SE-sIgE (kU/L)	Median 0.12 (IQR 0.04–0.37)	Median 0.09 (IQR 0.03–0.15)	Median 0.31 (IQR 0.13–0.91) ^{,*¶}	0.004 [§]
SE-sIgE ≥ 0.35 kU/L (%)	26.1	14.3	44.4	$< 0.001^{**}$

BMI, body mass index; SE-sIgE, staphylococcal enterotoxin-specific IgE; IQR, interquartile ranges.

See Table 1 for definition of current asthma, rhinitis, and inhalant allergen sensitization.

*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.

‡Adult-onset asthma was defined by a participant-recalled asthma starting after 18 years old.

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

^{||} $P < 0.05$ between never asthma and current asthma.

[¶] $P < 0.05$ between past asthma and current asthma by *post hoc* analyses.

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

Table 5. Multivariate logistic regression tests for adult-onset current asthma*

Adult-onset current asthma (<i>n</i> = 60) vs. never asthma (<i>n</i> = 989)	Adjusted OR (95% CI) [†]	<i>P</i> -value
Age	1.07 (1.04–1.11)	< 0.001
Sex		
Female	Reference	
Male	1.05 (0.44–2.52)	0.912
BMI (kg/m ²)		
< 23	Reference	
23.0–24.9	0.98 (0.45–2.13)	0.964
25.0–27.4	1.02 (0.45–2.32)	0.966
≥ 27.5	2.55 (1.11–5.84)	0.027
Smoking status		
Never smoker	Reference	
Ex-smoker	0.84 (0.33–2.15)	0.715
Current smoker	1.01 (0.39–2.60)	0.987
Alcohol status		
Never drinker	Reference	
Ex-drinker	2.65 (1.05–6.64)	0.039
Current drinker	0.84 (0.40–1.73)	0.629
Inhalant allergen sensitization		
No	Reference	
Yes	0.26 (0.06–1.13)	0.072
Rhinitis		
No	Reference	
Yes	2.02 (1.01–4.08)	0.049
SE-sIgE levels		
Negative (< 0.10 kU/L)	Reference	
Lowest tertile (0.10–0.22 kU/L)	2.38 (0.91–6.25)	0.078
Mid-tertile (0.22–0.61 kU/L)	5.07 (2.17–11.8)	< 0.001
Highest tertile (0.61–50.68 kU/L)	6.02 (2.49–14.5)	< 0.001

OR, odds ratio; CI, confidence interval; BMI, body mass index; SE-sIgE, staphylococcal enterotoxin-specific IgE.

Please see Tables 1 and 4 for definitions.

P-values were determined by multivariate logistic regression tests.

*Adult-onset current asthma was defined by current asthma (defined by current wheeze and methacholine airway hyperresponsiveness) with a participant-recalled onset after 18 years old.

[†]Adjusted for age, sex, BMI category, smoking status, alcohol status, inhalant allergen sensitization, and rhinitis.

recall bias, as the information was collected by questionnaires. The finding could also have been related to less symptomatic features of childhood-onset asthma compared to adult-onset asthma in older adults [20], or related to the characteristics of the study population (mean age: 60.2 years). Despite the shortcomings, we suggest a potential role of SE sensitization in the pathogenesis of adult-onset asthma, as the associations were independently significant.

Despite the significance of SE sensitization, the epidemiology is still underinvestigated. In the current literature, there are only a few studies to report the prevalence in community-based populations [14, 18, 19]. Nevertheless, it seems more clear that SE sensitization is prevalent

in the community; it ranged from 9.6% to 19.3% in paediatric populations (by ≥ the 0.35 kU/L cut-off) [18, 19] and was 29.3% in European adult populations (by ≥ 0.10 kU/L) [14]. Together with the present findings (27.0% by ≥ 0.35 kU/L, or 55.7% by ≥ 0.10 kU/L), we suggest the substantial burden of the sensitization in allergic disorders.

In this regard, risk factors for the sensitization need to be investigated. Interestingly, smoking was a major risk factor. The mechanisms of action are unclear, but cigarette smoke may promote allergen penetration into human bronchial epithelia and thus elicit allergen-specific responses from resident basophils [21]. 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. Another interesting finding was that positive effects of smoking on asthma risk disappeared when coadjusted with SE-sIgE levels in multivariate logistic regressions. These findings warrant further investigations; however, we postulate that the pathogenic role of smoking in adult asthma pathogenesis [2] is at least partly mediated by SE sensitization.

Male sex as a risk factor may seem unusual, considering that female sex has been more frequently associated with allergic diseases [22]; however, the findings were in line with the European adult population study [14]. As males had longer or more intense smoking histories than females, the male preponderance may have resulted from certain lifestyle factors such as smoking. However, in the present study, male sex and current smoking were associated with SE sensitization, independently from each other.

The relationship between older age (≥ 61 years) and SE sensitization is another interesting finding. As skin from older individuals has a reduced barrier function or an increased epidermal permeability to exogenous antigens [23], we presume that the risk of sensitization increases with ageing. Compared with previous population-based studies, the SE sensitization rate was significantly higher among our control subjects (mean age: 59.7 years, 26.1%) than among younger controls in the previous two studies (mean age: 14 years, 17.9% in Australian controls [19] and mean age: 5 years, 7.5% in UK children [18]). Although these data are not directly comparable, age could have an effect on SE sensitization, such that 'older age' may be a risk factor for the sensitization and for later developing asthma when combined by cofactors. In the literature, SE sensitization rate ranges from 38 to 76% of adult asthmatics [12], indicating the presence of specific asthma subtype mediated by SE sensitization.

We have previously shown that SE-sIgE is specifically related to severe non-atopic asthma in adults [7]. Collectively, we suggest a potential role of SE-sIgE in the pathogenesis of adult-onset non-atopic asthma among older adults.

However, there is no direct evidence for *S. aureus* enterotoxin pathogenesis in human airway disease, despite overwhelming experimental evidence in animal studies and human mucosal models that staphylococcal enterotoxins break tolerance [24] and induce airway inflammation and IgE synthesis [25–27]. Serum IgE levels correlate with local airway SE-sIgE levels or are functional [28]; however, serum SE-sIgE could be a surrogate marker that reflects high exposure to various microbial antigens, although this possibility has been partially excluded [19]. *S. aureus* and various other pathogens coexist in indoor dust [29], and high indoor levels of bacteria and moulds are correlated with asthma severity [30]. 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 [31, 32]. Therefore, these findings warrant further studies to directly demonstrate the causative role of *S. aureus* in the pathogenesis of asthma.

Our findings on the association with inhalant allergen sensitization confirm previous results from children/adolescent population studies [18, 19] and extend this knowledge into older adults. 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 an increased risk of bacterial survival [33]. Conversely, there is also experimental evidence that nasal staphylococcal enterotoxin B (SEB) administration enhances allergen sensitization in mice [24], and in *in vitro* studies using human dendritic cells (DC), SEB caused DCs to drive the Th2 polarization of naïve T cells [34]. Clinically, our cross-sectional findings could suggest that inhalant allergen sensitization during early life predisposes to the SE sensitization at later life, as they showed discordant patterns with regard to ageing (Table 2 and S3); however, further longitudinal studies are necessary to clarify the relationships.

Correlations between SE-sIgE and total IgE levels also need to be discussed. Total IgE, which was a traditional indicator of allergy or atopy, has been associated with demographic or environmental factors [35, 36], genetic factors [37], inhalant allergen sensitization [36], or parasitic infection [38]. We performed a comprehensive analysis of IgE in areas with high rates of endemic

Clonorchis infection to identify 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 potential effects of SE-sIgE need to be taken into consideration when interpreting total IgE levels, particularly in older adult populations. Considering the heterogeneity of adult-onset asthma and the significance of endotype approaches [39, 40], we feel that SE-sIgE is a useful biomarker to be included in clinical or epidemiological studies for adult-onset asthma.

The present study has several limitations. By using a cross-sectional survey, we could not determine causal relationships between the parameters. The results could not be generalized because the survey was not conducted nationwide. However, the prevalence of asthma was comparable to that in other community-based random population surveys in Korean older adults [16, 41]. Because the study participants were not randomly recruited but were volunteers from communities, selection biases may have existed. Recall biases also need to be taken into account, as our phenotype definition was partly based on self-administered questionnaires. However, the present study has strengths in that the demographic features were different from previous community population-based studies [14, 18, 19], and we included additional analyses by age of asthma onset. Thus, the present findings are new addition to previous knowledge.

In conclusion, we report the epidemiology and the significance of staphylococcal sensitization in the community-based adult populations. Our identification of risk factors for SE sensitization may be useful in understanding the pathophysiology of SE-mediated allergic diseases. The strong correlation between SE-sIgE and total IgE levels also warrants further investigation. The independent relationships with adult-onset asthma warrant further studies to clarify the precise pathogenic roles of staphylococcal sensitization.

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Conflict of interests

The authors declare that they have no relevant conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Univariate relationship between demographic parameters and staphylococcal enterotoxin sensitization.

Table S2. Relationships between inhalant allergen and staphylococcal enterotoxin sensitization.

Table S3. Multivariate logistic regression tests for inhalant allergen sensitization.

Supplementary material

Title: Staphylococcal enterotoxin sensitization in a community-based population: a potential role in adult-onset asthma

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Supplementary Table E1. Univariate relationship between demographic parameters and Staphylococcal enterotoxin sensitization

	SE sensitization% (≥ 0.35 kU/L)	<i>P</i> value
Residence area		
Sancheong, n=605	27.60	0.636
Changwon, n=475	26.32	
Age group		
≤ 40 yrs, n=83	14.5	0.041
41–50 yrs, n=153	24.2	
51–60 yrs, n=252	28.2	
61–70 yrs, n=374	30.0	
> 70 yrs, n=218	27.5	
Sex		
Female, n=678	17.55	< 0.001
Male, n=402	43.03	
BMI (kg/m ²)		
< 23 , n=486	27.16	0.521
23.0–24.9, n=285	24.56	
25.0–27.4, n=205	27.80	
≥ 27.5 , n=103	32.04	
Smoking		
Never smoker, n=679	17.23	< 0.001
Ex-smoker, n=191	35.08	
Current smoker, n=180	52.78	
Alcohol		
Never drinker, n=547	19.93	< 0.001
Ex-drinker, n=77	32.47	
Current drinker, n=427	33.96	
Diabetes mellitus		
No, n=985	26.1	0.024
Yes, n=95	36.8	
Inhalant allergen sensitization		
No, n=936	24.79	< 0.001
Yes, n=127	44.09	
Clonorchis infection		
No, n=778	24.94	0.003
Yes, n=264	34.47	

Abbreviations: SE, Staphylococcal enterotoxin; BMI, body mass index

Please see Table 1 for definitions.

P values were determined by chi-squared tests.

Supplementary Table E2. Relationships between inhalant allergen and Staphylococcal enterotoxin sensitization

	SE sensitization% (≥ 0.35 kU/L)	<i>P</i> value
Inhalant allergen sensitization		
No, n=937	24.9	< 0.001
Yes, n=127	43.3	
Number of sensitized inhalants		
0, n=937	24.9	0.002
1, n=72	40.3	
2, n=31	45.2	
3, n=12	50.0	
≥ 4 , n=12	50.0	

Abbreviation: SE, Staphylococcal enterotoxin

Please see Table 1 for definition of atopy.

P values were determined by likelihood-ratio chi-squared tests.

Supplementary Table E3. Multivariate logistic regression tests for inhalant allergen sensitization

	Adjusted OR (95% CI)*	P value
Residence area		
Sancheong	Reference	
Changwon	1.27 (0.83–1.94)	0.263
Age	0.95 (0.93–0.97)	< 0.001
Sex		
Female	Reference	
Male	1.94 (1.07–3.54)	0.029
BMI (kg/m ²)		
< 23	Reference	
23.0 – 24.9	0.94 (0.56–1.55)	0.801
25.0 – 27.4	1.11 (0.64–1.91)	0.711
≥ 27.5	1.28 (0.65–2.52)	0.482
Smoking		
Never smoker	Reference	
Ex-smoker	0.45 (0.22–0.95)	0.036
Current smoker	0.63 (0.32–1.23)	0.173
Clonorchis infection		
No	Reference	
Yes	1.81 (1.16–2.84)	0.010
SE-sIgE levels		
Negative (< 0.10 kU/L)	Reference	
Lowest tertile (0.10–0.22 kU/L)	1.40 (0.77–2.55)	0.275
Mid-tertile (0.22–0.61 kU/L)	1.60 (0.90–2.84)	0.107
Highest tertile (0.61–50.68 kU/L)	3.13 (1.79–5.48)	< 0.001

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

Please see Table 1 for definitions.

P values were determined by multivariate logistic regression tests.

* Adjusted for residence area, age, sex, BMI category, smoking status, clonorchis infection, and tertile-based SE-sIgE category