

A Distinct Sensitization Pattern Associated with Asthma and the Thymic Stromal Lymphopoietin (TSLP) Genotype

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ABSTRACT

Background: Atopy is a phenotypically heterogeneous condition, and the extent to which atopy accounts for asthma is controversial. In this study, we aimed to identify the presence of distinct sensitization patterns to common inhaled allergens and their association with asthma, allergic rhinitis and *TSLP* genotypes.

Methods: We studied 1683 adults from Tsukuba, a city in central Japan and 297 adults from Kamishihoro, a cedar-free, birch-dominant town in northern Japan. Levels of total serum IgE and specific IgE antibodies towards 14 major inhaled allergens were measured. With the use of these measures, cluster analysis was applied to classify the subjects' sensitization patterns. We also examined the genetic effects of 2 *TSLP* functional SNPs on the development of each sensitization pattern.

Results: In the Tsukuba study, cluster analysis identified four clusters, including "Dust mite dominant", "Multiple pollen", "Cedar dominant", and "Low reactivity". In the Kamishihoro study, "Dust mite dominant", "Multiple pollen" and "Low reactivity" clusters were also identified, but a "Cedar dominant" cluster was not formed. The association with asthma was strongest for the "Dust mite dominant" cluster in both the Tsukuba and the Kamishihoro studies. In never smokers, both SNPs were associated with the "Dust mite dominant" cluster (OR > 1.2). In contrast, in current or past smokers, these alleles were inversely associated with the "Multiple pollen" cluster (OR < 0.5).

Conclusions: Cluster analysis identified the presence of distinct sensitization patterns to common inhaled allergens. *TSLP* may cause asthma by promoting innate allergic responses to indoor allergens and this contribution is significantly modified by smoking.

KEY WORDS

cluster analysis, immunoglobulin E (IgE), sensitization pattern, smoking habit, *TSLP*

INTRODUCTION

Atopy is defined as a positive allergen-specific IgE antibody or skin prick test (SPT) to any common food or inhaled allergens. Overall, patients with asthma

have higher rates of sensitization to outdoor pollen allergens and indoor allergens such as house dust mite (HDM) than do non-asthmatics individuals. In the case of sensitization to the HDM allergen, the commonly accepted hypothesis is that exposure of geneti-

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cally susceptible individuals to allergen leads to the development of sensitization, and subsequently to asthma.¹ However, the association of allergic sensitization with asthma is not equivalent for all allergens: pollen allergy in asthmatics may be more an epiphenomenon, whereas indoor allergens are likely to be direct environmental causes of asthma.^{2,3}

Atopy is a phenotypically heterogeneous condition, and the extent to which atopy accounts for asthma is controversial. Several distinct phenotypes of atopy have been suggested. By adopting a machine learning approach in a large birth cohort study, Simpson *et al.* have demonstrated that children with atopic vulnerability are clustered into four distinct classes and that only one of these atopic classes predicts asthma, indicating that IgE antibody responses do not reflect a single phenotype of atopy.⁴ Dottorini *et al.*, using a microarray immunoassay containing 103 allergens, also studied the IgE reactivity profiles of 485 asthmatic and 342 nonasthmatic individuals and found that particular reactivity profiles were significantly associated with asthma.⁵ These findings indicate that asthma is a higher-order phenomenon related to patterns of IgE reactivity rather than to single antibody reactions.

Recent genome-wide association studies (GWAS) and meta-analyses of GWAS have begun to shed light on distinct pathways that contribute to asthma. Associations with variations in genes encoding epithelial cell-derived cytokines, interleukin-33 (IL-33) and thymic stromal lymphopoietin (TSLP) highlight the central roles for innate immune response pathways that promote the activation and differentiation of T helper type 2 (Th2) cells in the pathogenesis of asthma.⁶ Respiratory viruses, multiple protease allergens and inflammatory cytokines can induce TSLP expression in airway epithelial cells, and TSLP plays a critical role in sensing environmental agents and mediating Th2 cell responses following exposure.⁷ HDM-specific immune responses are initiated when allergen-associated danger signals are recognized by innate immune cells and pattern recognition receptors, leading to an increased expression of TSLP. The human *TSLP* gene is located on chromosome 5q22.1 next to the atopic cytokine cluster on 5q31.⁸ We recently identified the gene encoding *TSLP* as a strong susceptibility gene for adult asthma in a Japanese population.⁹

Given that more homogeneous atopy phenotypes may lead to a better understanding of the genetic risk factors for asthma and rhinitis, in this study, we used cluster analysis to identify distinctive characteristics associated with sensitization toward common inhaled allergens and then investigated how these different sensitization patterns are related to asthma, allergic rhinitis, or the functional polymorphisms located in the putative promoter regulatory regions of the *TSLP* gene that were previously shown to be associated

with asthma in Japanese individuals.⁹

METHODS

STUDY DESIGN

The cross-sectional survey was conducted in Tsukuba (a city in central Japan) and in Kamishihoro (a town in northern Japan), as previously described.¹⁰⁻¹² The characteristics of the two study populations are summarized in Table 1. Data on allergic symptoms, allergic diseases and cigarette smoking habit were assessed by a self-administered questionnaire. The diagnosis of asthma basically relied on a positive response to the following questions: "Have you ever had asthma?" followed by "Was this confirmed by a doctor?" in addition to the presence of asthmatic symptoms such as wheezing, cough, dyspnea, and chest tightness or the current use of anti-asthmatic medications. We classified subjects as having allergic rhinitis if they responded affirmatively to the question, "Do you have any nasal allergies including hay fever?" in addition to the presence of specific IgE antibody toward at least one inhaled allergen.

We classified subjects according to their smoking behavior. Current smokers were defined as those who currently smoke at least 1 cigarette a day or 1 cigarette a week for the past year. Former smokers were defined as those who had smoked for at least 1 year but not during the last month. All other subjects were considered as never-smokers. The smoking index for current and former smokers was calculated by multiplying the smoking dose (cigarettes per day) by the duration (years smoked).

The institutional review boards of the University of Tsukuba (IRB No. 136) and Tsukuba Medical Center (IRB No. 2008-01-31) approved the study, and each subject provided written informed consent.

IGE MEASUREMENT

Levels of total IgE were measured by the ImmunoCAP system (Phadia Ltd, Uppsala, Sweden). Specific serum IgE antibody was measured with the multiple allergen simultaneous test (MAST)-26 chemiluminescent assay systems (Hitachi Chemical Co., Tokyo, Japan).¹³ Fourteen major inhaled allergens including *Dermatophagoides farinae* (*D. farinae*), house dust, cat epithelium, dog epithelium, timothy grass, sweet vernal grass, ragweed mix, mugwort, Japanese cedar, *Penicillium*, *Cladosporium*, *Candida*, *Alternaria* and *Aspergillus* were selected. Birch specific serum IgE was additionally measured by ImmunoCAP in the Kamishihoro cohort. All sera were analyzed in the same laboratory in Tokyo. The cutoff values for the MAST assay and ImmunoCAP were 1.00 lumicount and 0.34 U_A/mL, respectively.

TSLP GENOTYPING

A total of 23 polymorphisms in the *TSLP* gene have been identified in the Japanese population.¹⁴ Among

Table 1 Baseline characteristics of the two study populations

Characteristic	Tsukuba study N = 1683	Kamishihoro study N = 297
Origin	Healthy adults who underwent medical checkups and asthmatic subjects living in the Tsukuba area	Kamishihoro residents; birch trees are dominant in this cedar-free area
Recruitment period	April 2008-March 2010	April 2006-March 2008
Age—yr, median (range)	51 (20-84)	50 (21-87)
Male sex—no. (%)	770 (45.7)	117 (39.4)
Smoking status—no. (%)		
Current smoker	220 (13.1)	67 (22.7)
Former smoker	386 (22.9)	72 (24.3)
Never smoker	1076 (64.0)	157 (53.0)
Smoking index [†]		
0	1077 (64.0)	157 (53.6)
0-200	188 (11.2)	35 (11.9)
>200	417 (24.8)	101 (34.5)
Asthma—no. (%)	266 (15.8)	52 (18.1)
Allergic rhinitis—no. (%)	749 (44.5)	115 (38.7)
Allergic sensitization—no. (%)	1010 (60.0)	158 (53.1)
Number of positive sera—means $\pm$ SD	1.66 $\pm$ 2.28	1.98 $\pm$ 2.97
Total serum IgE, median (range) (IU/ml)	66.9 (5.0-32000)	79 (2.5-7540)

[†]Smoking index was calculated for current and former smokers by multiplying smoking dose (cigarettes per day) and duration (years smoked).

3 tag SNPs (rs3806933, rs2289276, and rs2289278), two functionally relevant SNPs in *TSLP*, rs3806933 and rs2289276, were genotyped using the TaqMan allele-specific amplification method (Applied Biosystems, Foster City, CA, USA) as described previously.^{10,14} We chose these 2 SNPs for analyses because, in our previous studies, these 2 SNPs were associated with the higher promoter activity of the gene and with the diagnosis of asthma^{9,14} and with the decreased lung function in Japanese non-COPD, non-asthmatic healthy individuals.¹⁰

Genomic DNA was available for genotyping in a total of 1806 subjects (1618 Tsukuba residents and 188 Kamishihoro residents).

CLUSTER ANALYSIS AND DISCRIMINANT ANALYSIS

A uniform cluster analysis methodology was applied to each population. In the first step, hierarchical cluster analysis using Ward's method generated a dendrogram for estimation of the number of likely clusters within the studied population. In the second step, a *k*-means cluster analysis was repeated with the pre-specified clusters and the most representative model was selected in each population. Variables included log-transformed standardized total IgE and specific IgE concentrations toward 14 common inhaled allergens (lumicount). To evaluate the input variables that were significant determinants of cluster structure, linear discriminant analysis was performed using both

forward and backward stepwise algorithms for each cluster model. The selection of variables was made according to the following criteria: larger *F* ratios (more than 1/10 of the largest *F*) and smaller *P* values.

STATISTICAL METHODS

The χ^2 test was used to compare categorical covariates, and the Wilcoxon rank sum test to compare the continuous variables of age, number of positive sera and serum IgE levels. Multivariate logistic regression analyses were performed to relate asthma or allergic rhinitis with sensitization clusters adjusting for potential confounders such as age, sex, smoking and the presence of rhinitis or asthma. The results were expressed in adjusted odds ratios (OR), "Low reactivity" cluster being the reference. The proportion of asthma or allergic rhinitis cases that were attributable to each cluster were also estimated by the formula $P(OR-1)/OR$, where *P* is the percentage of disease cases with the risk factor.¹⁵ The population attributable risk (PAR) was adjusted using the adjusted OR. In addition, we examined the genetic effects of *TSLP* on the development of sensitization patterns using multinomial logistic regression analysis. For this genetic analysis, we studied two populations together, dividing the subjects into two groups (never smokers and former or current smokers) because smoking is known to be a strong inducer of *TSLP* in the airways.¹⁶ We also tested the presence of an interaction

Table 2A Characteristics of subjects according to classifications by clusters in Tsukuba study

	Dust mite dominant	Multiple pollen	Cedar dominant	Low reactivity	P-value
N	174	66	313	1130	
Age—yr, median (range)	42 (20-67)	47 (30-61)	48 (25-74)	53 (23-84)	<0.0001
Male sex—no. (%)	94 (54.0)	34 (51.5)	122 (38.9)	520 (46.0)	0.0093
Smoking status—no. (%)					0.49
Current smoker	27 (15.5)	7 (10.6)	38 (12.1)	148 (13.1)	
Former smoker	43 (24.7)	21 (31.8)	67 (21.4)	255 (22.5)	
Never smoker	104 (59.7)	38 (57.7)	208 (66.5)	726 (64.3)	
Smoking index [†]					<0.0001
0	105 (60.3)	38 (57.6)	208 (66.5)	726 (64.3)	
0-200	29 (16.7)	20 (30.3)	43 (13.7)	96 (8.5)	
>200	40 (23.0)	8 (12.1)	62 (19.8)	307 (27.2)	
Asthma—no. (%)	73 (42.0)	11 (16.7)	44 (14.1)	138 (12.2)	<0.0001
Allergic rhinitis—no. (%)	93 (53.7)	55 (83.3)	273 (87.2)	328 (29.0)	<0.0001
Allergic sensitization—no. (%)	174 (100.0)	66 (100.0)	313 (100.0)	457 (40.4)	<0.0001
Number of positive sera—means $\pm$ SD	4.81 $\pm$ 2.27	5.84 $\pm$ 3.21	2.35 $\pm$ 1.97	0.73 $\pm$ 1.31	<0.0001
Total serum IgE, median (IQR) (IU/ml)	292.5 (123.0-685.3)	263.5 (101.4-577.8)	142.0 (70.5-271.0)	40.3 (18.2-96.0)	<0.0001

[†]Smoking index was calculated for current and former smokers by multiplying smoking dose (cigarettes per day) and duration (years smoked). IQR, interquartile range.

Table 2B Characteristics of subjects according to classifications by clusters in Kamishihoro study

	Dust mite dominant	Multiple pollen	Cedar dominant	Low reactivity	P-value
N	53	16	0	228	
Age—yr, median (range)	34 (21-71)	35 (27-60)		56 (21-87)	<0.0001
Male sex—no. (%)	19 (35.8)	7 (43.8)		91 (39.9)	0.81
Smoking status—no. (%)					0.18
Current smoker	15 (28.3)	4 (25.0)		48 (21.1)	
Former smoker	6 (11.3)	4 (25.0)		62 (27.3)	
Never smoker	32 (60.3)	8 (50.0)		117 (51.5)	
Smoking index [†]					<0.0001
0	32 (60.4)	8 (53.3)		117 (52.0)	
0-200	4 (7.6)	3 (20.0)		28 (12.4)	
>200	17 (32.0)	4 (26.7)		80 (35.6)	
Asthma—no. (%)	21 (39.6)	3 (18.7)		30 (13.1)	<0.0001
Allergic rhinitis—no. (%)	28 (52.8)	8 (50.0)		79 (34.6)	0.032
Allergic sensitization—no. (%)	15 (28.3)	4 (25.0)		27 (11.8)	0.0065
Number of positive sera—means $\pm$ SD	5.07 $\pm$ 3.42	7.76 $\pm$ 4.16		0.89 $\pm$ 1.49	<0.0001
Total serum IgE, median (IQR) (IU/ml)	310 (119.5-681.0)	348.5 (79.5-861.3)		51.2 (20.8-155.3)	<0.0001

[†]Smoking index was calculated for current and former smokers by multiplying smoking dose (cigarettes per day) and duration (years smoked). IQR, interquartile range.

between *TSLP* genotypes and smoking status on the development of sensitization patterns.

All statistical analyses of the data were performed using JMP 9 Windows statistics software (SAS Institute Inc., Cary, NC, USA). *P* values less than 0.05 were considered significant.

RESULTS

In the first population (Tsukuba), the cluster analysis identified four well-separated clusters (Table 2A).

The “Dust mite dominant” cluster (174/1683, 10.3%) was characterized by strong sensitization to indoor allergens, specifically *D. farinae*, and higher levels of total serum IgE. Subjects in the “Multiple pollen” (66/1683, 3.9%) were strongly sensitized to many types of pollens, particularly timothy grass. Subjects in the “Cedar dominant” cluster (313/1683, 18.6%) were strongly sensitized to cedar pollen only. Subjects in the “Low reactivity” cluster (1130/1683, 67.1%) were not strongly sensitized to any of the com-

Table 3A Association of asthma with sensitization patterns

	Tsukuba study (<i>N</i> = 1683)			Kamishihoro study (<i>N</i> = 297)		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Covariates						
Sex (female/male)	1.36	0.82-2.27	0.232	1.90	0.87-4.29	0.115
Age	1.01	0.98-1.03	0.548	1.03	1.00-1.05	0.038
Smoking status						
Current/Never	1.21	0.66-2.15	0.532	2.29	0.98-5.39	0.717
Former/Never	1.02	0.51-1.93	0.957	1.20	0.44-3.11	0.056
Allergic rhinitis (+/-)	1.23	0.73-2.05	0.436	2.13	1.12-4.09	0.021
Cluster						
Dust mite dominant/Low reactivity	7.90	4.46-13.98	<0.0001	5.73	2.55-13.39	<0.0001
Multiple pollen/Low reactivity	2.86	1.06-6.88	0.026	2.08	0.43-7.73	0.306
Cedar dominant/Low reactivity	1.54	0.78-2.99	0.204	NA	NA	

OR, odds ratio; 95% CI, 95% confidence interval; NA, not applicable.

Table 3B Association of allergic rhinitis with sensitization patterns

	Tsukuba study (<i>N</i> = 1683)			Kamishihoro study (<i>N</i> = 297)		
	OR	95% CI	<i>P</i> value	OR	95% CI	<i>P</i> value
Covariates						
Sex (female/male)	0.88	0.65-1.19	0.421	0.93	0.50-1.71	0.82
Age	0.98	0.96-0.99	0.0017	0.99	0.97-1.01	0.166
Smoking status						
Current/Never	0.69	0.48-0.99	0.049	0.51	0.25-1.00	0.05
Former/Never	0.78	0.52-1.16	0.22	0.49	0.23-1.01	0.057
Asthma (+/-)	1.24	0.74-2.06	0.405	2.19	1.15-4.19	0.017
Cluster						
Dust mite dominant/Low reactivity	4.21	2.82-6.31	<0.0001	1.34	0.66-2.72	0.419
Multiple pollen/Low reactivity	22.67	11.47-50.23	<0.0001	1.52	0.51-4.54	0.447
Cedar dominant/Low reactivity	30.28	20.61-45.80	<0.0001	NA	NA	NA

OR, odds ratio; 95% CI, 95% confidence interval; NA, not applicable.

mon inhaled allergens. The variables that were most significant determinants of the clusters were *D. farina* (*F*-value; 2925.5), timothy grass (2246.1) and Japanese cedar (971.0). The strongest association of allergen sensitization with asthma was observed in the “Dust mite dominant” cluster with an OR of 7.90 (95% CI: 4.46-13.98) (Table 3A). The highest independent risk for rhinitis was “Cedar dominant” sensitization (OR: 30.28; 95% CI: 20.61-45.80) (Table 3B). The proportion of asthma attributable to “Dust mite”, “Multiple pollen” or “Cedar dominant” cluster is 30.2%, 4.8%, or 8.5%, respectively. The proportion of allergic rhinitis attributable to “Dust mite”, “Multiple pollen” or “Cedar dominant” is 16.8%, 13.7% or 43.9%, respectively.

In the second population (Kamishihoro), “Dust mite dominant” (53/297, 17.8%), “Multiple pollen” (16/297, 5.4%) and “Low reactivity” (228/297, 76.8%) clusters were also identified, whereas a “Cedar dominant” was not formed presumably because the

Kamishihoro is a cedar free city (Table 2B). When compared with the “Low reactivity” cluster, the association with asthma was also strongest for the “Dust mite dominant” cluster (OR: 5.73, 95% CI: 2.55-13.39) (Table 3A). The proportion of asthma attributable to “Dust mite” or “Multiple pollen” cluster is 34.7% or 4.7%, respectively. The proportion of allergic rhinitis attributable to “Dust mite” or “Multiple pollen” is 6.6% or 3.1%, respectively.

In never smokers, both at rs3806933 and rs2289276, the *TSLP* alleles linked to higher promoter activity of the gene and with diagnosis of asthma^{9,14} were significantly associated with the “Dust mite dominant” cluster (OR 1.42 [1.03-1.96]; *P* = 0.033, OR 1.43 [1.03-1.99]; *P* = 0.033, respectively) (Table 4A, B). In contrast, in current or former smokers, these alleles were inversely associated with the “Multiple pollen” cluster (OR 0.42 [0.21-0.85]; *P* = 0.015, OR 0.38 [0.18-0.80]; *P* = 0.011, respectively) (Table 4A, B). For the “Dust mite dominant” cluster,

Table 4A Genetic influence of *TSLP* (rs3806933) on atopy clusters in smokers and non-smokers

	Never smokers (N = 1164)			Current or former smokers (N = 641)		
	OR	95% CI	P value	OR	95% CI	P value
Low reactivity (n = 1250)	Reference	-	-	Reference	-	-
Cedar dominant (n = 298)	1.05	0.81-1.34	0.73	0.85	0.61-1.19	0.35
Multiple pollen (n = 71)	1.25	0.77-2.03	0.37	0.42	0.21-0.85	0.015
Dust mite dominant (n = 186)	1.42	1.03-1.96	0.033	0.70	0.47-1.05	0.08

For the Dust mite dominant cluster, P value for interaction between *TSLP* genotype and smoking status was 0.014.

OR, odds ratio; 95% CI, 95% confidence interval.

Table 4B Genetic influence of *TSLP* (rs2289276) on atopy clusters in smokers and non-smokers

	Never smokers (N = 1164)			Current or former smokers (N = 639)		
	OR (95%CI)	95% CI	P value	OR	95% CI	P value
Low reactivity (n = 1250)	Reference	-	-	Reference	-	-
Cedar dominant (n = 298)	1.06	0.82-1.38	0.63	0.84	0.60-1.19	0.33
Multiple pollen (n = 71)	1.00	0.59-1.70	0.99	0.38	0.18-0.80	0.011
Dust mite dominant (n = 184)	1.43	1.03-1.99	0.033	0.77	0.52-1.15	0.20

For the Dust mite dominant cluster, P value for interaction between *TSLP* genotype and smoking status was 0.032.

OR, odds ratio; 95% CI, 95% confidence interval.

the P values for interaction between *TSLP* genotype and smoking status were 0.014 and 0.032 for rs3806933 and rs2289276, respectively.

DISCUSSION

Atopy, or allergen-specific IgE responsiveness, is a phenotypically heterogeneous condition. Identification of more homogeneous phenotypes of atopy may lead to better understanding of their importance as etiologies of asthma and rhinitis. In this study, cluster analysis with total serum IgE and IgE specific to 14 inhaled allergens was used to identify phenotypic patterns in two independent Japanese populations. In the original "Tsukuba" population, 4 clusters were identified: "Dust mite dominant", "Multiple pollen", "Cedar dominant", and "Low reactivity". These clusters were replicated in the second "Kamishihoro" population except for the "Cedar dominant" cluster. We then demonstrated that the "Dust mite dominant" cluster was significantly related to the development of asthma, whereas "Cedar dominant" clusters were greatly related to allergic rhinitis. Therefore, our results are in line with the contention that for asthmatics, sensitization to outdoor allergens such as grass pollens is not a risk factor for disease, but merely a marker of the atopic diathesis, while sensitization to dust mite allergens are more likely to be an intermediate step between allergen exposure and development of asthma.

TSLP is an established susceptibility gene for asthma across different ethnicities.⁶ Although *TSLP* is a key cytokine in initiation and maintenance of Th2-type allergic inflammation, its function is innate and not allergen-specific. Allergen-specific immune re-

sponses are initiated when allergen-associated danger signals are recognized by innate immune cells and pattern recognition receptors.^{17,18} In response to cysteine proteases, *TSLP* mediated by TLR4 and the adaptor TRIF is induced in epithelial cells, which drives Th2 immunity.¹⁹ *TSLP* expression is also induced in airway epithelial cells by exposure to allergen-derived proteases and PAR-2 is involved in the process.²⁰ Given these mechanisms, we associated the *TSLP* genotype with sensitization patterns rather than with a specific IgE response to each allergen. We found that the *TSLP* genotype was associated with the "Dust mite dominant" cluster, which may suggest that, at least in never smokers, *TSLP* causes asthma by promoting allergic innate responses to mite allergens and subsequently inducing Th2 inflammation in the asthmatic airways. A cluster, which describes "observable characteristics," does not necessarily relate to or give any insight into the underlying disease processes that is defined by a distinct functional or pathophysiological mechanism, and the presence of genetic association of *TSLP* genotype with the "Dust mite dominant" cluster indicates that the clusters identified in this study could represent clinically and biologically meaningful phenotypes of atopy.

The current study showed that the contribution of *TSLP* genotype to innate allergic responses is significantly modified by smoking. Recently, Liu *et al.* reported that a genetic variant in *TSLP* was associated with asthma in admixed urban populations in the United States.²¹ Very interestingly, they also found the presence of an environmental interaction: stronger association between *TSLP* and asthma was

noted in individuals with a history of smoking, indicating the complex interaction between genetic impacts of *TSLP* and smoking. Increasing evidence suggests that cigarette smoking adversely affects immunity.²² The consequences of this altered immune status on immune and inflammatory processes elicited by environmental agents, such as aeroallergens, are not well-understood. Therefore, it is important to note that in smokers, *TSLP* genotypes were inversely associated with the development of the "Multiple pollen" cluster. *TSLP* is a sensor of exogenous stimuli including smoking. In fact, *TSLP* is increased in the airways of patients with COPD,²³ suggesting that *TSLP* not only plays a key role in allergic inflammation, but also may play a role in nonallergic airway inflammation. Recent studies propose a novel role for *TSLP* in regulating the balance of airway tolerance and allergic inflammation²⁴; inhibition of inducible Treg (iTreg) differentiation may be an important component of the function of *TSLP*, suppressing airway tolerance and initiating allergen sensitization.²⁵ Since tobacco smoke includes a variable amount of endotoxin, which augments antigen-induced Th1 cells, some smokers susceptible to increased expression of *TSLP* may tend to have greater Th1 response through a greater down-regulation of regulatory T cells, leading to COPD and/or the inhibition of allergic sensitization and rhinitis.

A clear-cut difference has been reported between asthma and rhinitis in their associations with atopy and total serum IgE levels. Using 2657 subjects in a general population, Burrows *et al.* found that asthma is closely associated with increased levels of total serum IgE irrespective of atopic status, while allergic rhinitis is associated primarily with skin-test reactions to common aeroallergens.²⁶ In addition, many genetic studies aimed at identifying the genes underlying asthma and relevant traits recently showed that the genes controlling IgE levels have surprisingly little overlap with the genes mediating asthma susceptibility, suggesting that atopy is secondary to asthma rather than a primary driver of the disease.^{27,28} Therefore, it is also possible that the results of the current study just reflect *TSLP* gene as a gene that confers susceptibility to asthma itself rather than to increased IgE responsiveness or atopy.

Several studies have unraveled strong relationships among exposure to HDM, the presence of serum IgE directed against the mite allergens and asthma.²⁹ However, a large number of populations worldwide, even those living in regions with low lifetime exposure to mite antigens, do not show any decrease in the prevalence of asthma.³⁰ Therefore, while our study demonstrates a link between a specific sensitization pattern and asthma, it should be emphasized that a comprehensive understanding of the relationship between asthma and allergen-specific IgE would require an exhaustive analysis of specific IgE profiles

in populations exposed to different sets of allergens. In addition, delivery of allergens to the nose is exclusively on particles, which carry a range of molecules in addition to protein allergens. These molecules include pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), which can alter the immune response to allergens. There is also recognition that innate and adaptive immune functions decline with aging. In fact, there is a consistent observation that measures of allergic sensitization, such as skin prick testing, specific IgE, and total IgE, decline with age.³¹ Thus, the allergens required to understand the relationship with asthma probably vary dependent on both the geographical area, including rural and suburban areas, and aging.

In conclusion, four clusters identified in this study clearly discriminated populations in terms of allergen-specific IgE responsiveness towards common inhaled allergens. The *TSLP* genotype may cause asthma by enhancing innate responses to indoor allergens such as HDM, and participating in a gene and environmental interaction with smoking. Our multidimensional approach allows validation by replication across different populations and may contribute to a more reliable classification of allergen-specific IgE responsiveness and to improved precision in research relying on phenotype recognition, particularly in the field of genetics.

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