



Association of atopy phenotypes with new development of asthma and bronchial hyperresponsiveness in school-aged children

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ABSTRACT

Background: Although previous studies have investigated the association between atopy phenotypes and allergic diseases, atopy characterizations in association with the development of allergic diseases remain poorly understood.

Objective: To identify atopy phenotypes in school-age children and to evaluate the association between atopy phenotypes and allergic diseases.

Methods: We enrolled 616 children with atopy defined as 1 or more positive allergen responses on skin prick tests and 665 children without atopy from the Children's Health and Environmental Research (CHEER) study. All children were followed up for 4 years at 2-year intervals. Atopy phenotypes were classified using latent class analysis.

Results: Four atopy phenotypes were characterized: later sensitization to indoor allergens (cluster 1); multiple early sensitization (cluster 2); early sensitization to outdoor allergens, especially *Alternaria*, and later sensitization to indoor allergens, including *Aspergillus* (cluster 3); and early sensitization to indoor allergens and later sensitization to outdoor allergens (cluster 4). New cases of asthma during follow-up were increased in clusters 2 and 3 (adjusted odds ratio [aOR], 2.76 and 4.25, respectively). The risk of new-onset bronchial hyperresponsiveness was highest in cluster 3 (aOR, 5.03). Clusters 2 and 4 had an increased risk of allergic rhinitis (aOR, 7.21 and 2.37, respectively).

Conclusion: Identification of atopy phenotypes facilitates prediction of the development of asthma and bronchial hyperresponsiveness in school-age children. Our study suggests prevention of additional sensitization is required to modify the progression of allergic diseases.

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Introduction

The prevalence of allergic diseases, such as asthma, allergic rhinitis (AR), and atopic dermatitis (AD), has been increasing worldwide during the past few decades.^{1–4} Along with these allergic diseases, the prevalence of atopy has also been increasing and is considered a strong risk factor for the development of allergic diseases.^{5,6}

Atopy, generally defined as 1 or more positive responses on skin prick tests (SPTs) or serum specific IgE, has dynamic changes

according to age.⁷ Moreover, the sensitization timing (eg, early or late), sensitization number (eg, monosensitization or polysensitization), changes in sensitization degree (eg, increasing, decreasing, or flattening), and prognosis (eg, remission or persistence) vary among individuals.^{7–9} In addition to atopy itself, the heterogeneous features of atopy might be crucially attributable to the development and prognosis of allergic diseases through complex interactions between genetic and environmental factors.^{10,11}

Previous studies on atopy phenotypes using latent class analysis (LCA) have yielded diverse results.^{6,12,13} Highly sensitized children at 2 years of age were more susceptible to AD, asthma, and wheezing in a birth cohort study conducted in the United States.¹² Multiple early sensitizations increased the risk of asthma, up to the age of 8 years, in a birth cohort study from the United Kingdom.⁶ In a high-risk population-based study, the associations between atopy and allergic diseases were found to be diverse, according to the participant's age, sensitized allergens, and methods of atopy assessment.¹⁴ Although atopy is closely related to the development of allergic diseases other than asthma,^{15–17} most previous studies have focused on the association between atopy and asthma and/or lung function.^{6,12,14} Moreover, these previous studies had certain limitations, such as the small number of allergens assessed and the lack of the repetitive objective evaluation of asthma.^{6,12,13} Sensitization patterns exhibit dynamic changes in life, even in the same individual; hence, the repetitive assessment of atopy to common and different types of allergens is required to evaluate the wide spectrum of atopy phenotypes.

During childhood, sensitization patterns show a more diverse fluctuation, which might affect the development of allergic diseases in childhood, with more complex interactions than in younger age groups.^{7,16} To assess the mechanisms through which atopy contribute to the development of allergic diseases, we identified atopy phenotypes using LCA, which is a statistical method used to separate subgroups according to their similar characteristics on the basis of unbiased observed heterogeneity.¹⁸ In addition, we elucidated their associations with the development of diverse allergic diseases, including AD, AR, asthma, and bronchial hyper-responsiveness (BHR) in school-age children in a general population-based prospective study.

Methods

Study Population

We enrolled 616 children from the Children's Health and Environmental Research (CHEER) study, a general population-based prospective study conducted during 4 years with 2-year intervals that originally aimed to evaluate the effect of environmental factors on health in children.^{19,20} Children with available SPT results at every survey from 2005 to 2010 were enrolled; these children had a mean age of 7.4 years. An additional 665 children from the CHEER study, who were characterized as nonatopic at each survey, were included as controls. All the included children were prospectively followed up via questionnaires, blood tests, pulmonary function tests, methacholine challenge tests, and SPTs at each survey.^{19,20} The Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to investigate the presence of allergic diseases.^{1,3,21} Written informed consent was obtained from the parents or guardians of the study participants, and the study protocols were reviewed and approved by the institutional review board of Ulsan University College of Medicine. The parents or guardians completed the questionnaire.

Definitions of Asthma, AD, AR, and Food Allergy

A participant was considered to have asthma at enrollment if there was an affirmative response on the ISAAC questionnaire to

the following question, "Have you ever been diagnosed with asthma by a physician?" Current asthma was defined as physician-diagnosed asthma reported by the parents or guardians in the lifetime and the presence of asthma symptoms within the preceding 12 months during the study. AD was defined according to the parent-reported physician's diagnosis using the question, "Has your child been diagnosed with AD by a physician?" AR is usually defined as the presence of sneezing, runny nose, or nasal stuffiness unrelated to a respiratory infection. We considered physician-diagnosed AR according to parent-reported physician's diagnosis using the question, "Has your child been diagnosed with AR by a physician?" The presence of food allergy was defined according to the parents' response to the following question, "Has your child been diagnosed with a food allergy by a physician?"

New-onset asthma was defined as a change in the patient's history from no history of physician-diagnosed asthma at enrollment to the presence of physician-diagnosed asthma during the prospective follow-up period. New-onset AR was defined as a conversion from no history of physician-diagnosed asthma at enrollment to the presence of physician-diagnosed AR during the follow-up period. New-onset BHR was defined as a conversion from a negative BHR test result (>8 mg/mL) at enrollment to a positive BHR test result (≤ 8 mg/mL) during the prospective 4-year follow-up period.

SPTs

SPTs were performed for the 18 most common inhalant aeroallergens (Allergopharma GmbH & Co, Reinbek, Germany), including *Dermatophagoides pteronyssinus* (*D pteronyssinus*), *Dermatophagoides farinae* (*D farinae*), dog dander, cat epithelium, cockroach, *Alternaria alternata*, *Aspergillus fumigatus*, grass pollen mixture, tree mixture I (alder, elm, hazel, poplar, and willow), tree mixture II (beech, birch, oak, and plane tree), ragweed, mugwort, alder, and oak, and 4 food allergens, including peanuts, egg whites, cow's milk, and soybeans.^{19,20} Histamine (10 mg/mL) was used as the positive control, and normal saline was used as the negative control. A mean wheal size of 3 mm or larger for allergens and histamine when measured after 15 minutes was considered a positive result. Atopy was defined as the presence of 1 or more positive test results to any of the 22 allergens assessed using SPTs.^{19,20}

Pulmonary Function Test and BHR

Baseline lung function parameters, including forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and forced expiratory flow at 25% to 75% of FVC (FEF_{25%–75%}), were assessed according to the American Thoracic Society/European Respiratory Society guidelines.^{3,11,20} BHR was assessed on the same day in all the participants via a methacholine bronchial challenge using the 5-breath dosimeter method (0.625, 1.25, 2.5, 5, 10, and 25 mg/mL, in that order). The cumulative methacholine dose that caused a 20% reduction in FEV₁ (PC₂₀) according to the American Thoracic Society recommendations was recorded.²² Participants were considered to have BHR to methacholine when the methacholine PC₂₀ was 8 mg/mL or less.

Statistical Analysis

To perform LCA, demographic characteristics (such as sex and body mass index [BMI]), socioeconomic status (such as monthly income and maternal educational levels), environmental factors (such as exposure to environmental tobacco smoke), and SPT results (such as positive numbers on SPTs and sensitized allergens) were used.¹⁹ To determine the optimal number of clusters, we selected the lowest Bayesian information criterion,¹⁸ which was used to define 4 atopy clusters in this study. The sensitized

allergens were divided into the following 3 subtypes: indoor allergens, outdoor allergens, and food allergens.

The associations between comorbidities and atopy clusters were analyzed using multivariate logistic regression analysis and were expressed as adjusted odds ratios (aORs) with their 95% confidence intervals (CIs). Adjustments were made for potential confounding factors, including sex, age, BMI, household income, and parental history of allergic diseases. We assessed the significance using χ^2 , Fisher exact, or Kruskal-Wallis tests, as appropriate. All statistical analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina). $P < .05$ was considered statistically significant.

Results

Characteristics of the Study Population

The baseline characteristics of the study population are summarized in Table 1. The mean age of the 4 atopy phenotypes was 7.35 years, and 42.0% of the children in the 4 atopy clusters had parental allergic diseases. At enrollment, 57.5% of the children in the 4 atopy clusters had positive response on SPTs.

Characteristics of the Atopy Phenotypes

A total of 665 (51.9%) of the 1,281 study participants were in the control group (nonatopy). All these individuals had no atopy during the entire study period. Compared with participants in atopy phenotypes, the proportion of males was significantly lower in this group (297 [44.7%] of 665; $P = .001$) (Table 2).

A total of 262 (20.5%) of the 1,281 study participants were in cluster 1 (later sensitization to indoor allergens). All these participants were nonatopic at enrollment (Fig 1A). The most commonly sensitized allergens during the prospective follow-up were *D pteronyssinus* (176 [67.2%]) and *D farinae* (165 [63.0%]), followed by cockroach (32 [12.2%]) and *Alternaria* (24 [9.2%]) (Fig 1B).

Participants in this cluster had lower rates of high maternal educational levels and a parental history of allergic diseases (Table 2).

A total of 53 (4.1%) of the 1,281 study participants were in cluster 2 (early-onset multiple sensitization). All these individuals had sensitization to at least 1 indoor allergen, and 81.1% were simultaneously sensitized to at least 1 outdoor allergen at enrollment. In addition, all participants in this group had sensitization to at least 2 allergens, regardless of the outdoor or indoor allergens detected at enrollment. Participants in this group were most commonly sensitized to *D farinae* (49 [92.5%]) and *D pteronyssinus* (48 [90.6%]). *Alternaria* (22 [41.5%]) was the most commonly sensitized outdoor allergens at enrollment, followed by *Aspergillus* and mugwort (9 [17.0%] in both groups) (Fig 1A). Participants in this cluster had significantly higher levels of total serum IgE and blood eosinophil percentages at enrollment (Table 2).

A total of 35 (2.7%) of 1,281 study participants were in cluster 3 (early sensitization to outdoor allergens, especially *Alternaria*, and later sensitization to indoor allergens, including *Aspergillus*). All these individuals had sensitization to outdoor allergens at enrollment (Fig 2A). The most commonly sensitized allergens during follow-up were *Alternaria* (24 [68.6%]), followed by tree mix II and grass (6 [17.1%] in both groups). Participants in this cluster at enrollment were most commonly sensitized to *D pteronyssinus* (5 [14.3%]) and *D farinae* (9 [25.7%]), with increases in sensitization to tree allergens during the prospective follow-up (Fig 1B). The prevalence of new sensitization to *Alternaria* (2 [5.7%]) and tree mix II (6 [17.1%]) in this cluster was the highest across atopy phenotypes during the study period (Fig 1B). Participants in this cluster had higher levels of total serum IgE in combination with relatively low percentages of blood eosinophils (Table 2). The prevalence of sensitization to food allergens was highest in cluster 3, both at enrollment and at follow-up (Fig 2C).

A total of 266 (20.8%) of the 1,281 study participants were in cluster 4 (early sensitization to indoor allergens followed by outdoor allergens). All participants in this cluster were sensitized to

Table 1
Baseline Characteristics of the Study Population^a

Variable	Participants in 4 clusters (n = 616)	Excluded in LCA (n = 1875)	Participants in 4 clusters and control (n = 1281)	Excluded in this study (n = 1210)
Male	348 (56.5) ^b	949 (50.6)	645 (50.4)	652 (53.9)
Age, mean (SD), y	7.35 (0.41) ^b	7.78 (1.25)	7.35 (0.43) ^c	8.01 (1.47)
BMI, mean (SD)	16.81 (2.36)	16.91 (2.62)	16.71 (2.37) ^c	17.06 (2.73)
Parental allergic diseases, yes	256/609 (42.0) ^b	423/1,732 (24.4)	482/1,265 (38.1) ^c	197/1,076 (18.3)
Maternal educational level				
High school or less	204/556 (36.7)	591/1633 (36.2)	418/1,158 (36.1)	377/1,031 (36.6)
Parental monthly income, \$US				
≤3,000	196/603 (32.5)	513/1,759 (29.2)	369/1,262 (29.2)	340/1,100 (30.9)
ETS exposure, yes	249/548 (45.4)	767/1,683 (45.6)	533/1,159 (46)	483/1,072 (45.1)
Atopy on skin prick tests	354/616 (57.5) ^b	333/1,872 (17.8)	354/1,281 (27.6)	333/1,207 (27.6)
No. of sensitized allergens				
0	262/615 (42.6) ^b	1533/1,866 (82.2)	926/1,279 (72.4)	869/1,202 (72.3)
1	113/615 (18.4) ^b	115/1,866 (6.2)	113/1,279 (8.8)	115/1,202 (9.6)
2	174/615 (28.3) ^b	158/1,866 (8.5)	174/1,279 (13.6)	158/1,202 (13.1)
≥3	66/615 (10.7) ^b	60/1,866 (3.2)	66/1,279 (5.2)	60/1,202 (5)
Total serum IgE, mean (range), IU/mL	435.7 (3–5,521) ^b	214.8 (1.4–6,934)	282.4 (2.4–5,521)	256.3 (1.4–6,934)
Blood eosinophils, mean (range), %	4.55 (0–39.1) ^b	3.38 (0–22.6)	3.75 (0–39.1)	3.58 (0.2–20.4)
Asthma diagnosis, ever	89/578 (15.4) ^b	146/1,753 (8.3)	135/1,213 (11.1)	100/1,118 (8.9)
AR diagnosis, ever	184/577 (31.9) ^b	385/1,738 (22.1)	298/1,207 (24.7)	271/1,108 (24.5)
AD diagnosis, ever	211/577 (36.6) ^b	530/1,731 (30.6)	388/1,201 (32.3)	353/1,107 (31.9)
FEV ₁ , % predicted	105.8 (13.62)	105.7 (12.90)	106 (13.45)	105.4 (12.68)
FEV ₁ /FVC	90.35 (5.64)	90.77 (5.61)	90.87 (5.51)	90.45 (5.73)
FEF _{25%–75%} , % predicted	98.06 (22.36)	99.56 (22.80)	99.2 (22.54)	99.07 (22.9)
BHR (PC ₂₀ ≤ 8 mg/mL)	151/597 (25.3) ^b	337/1,828 (18.4)	262/1,245 (21)	226/1,180 (19.2)

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; BHR, bronchial hyperresponsiveness; BMI, body mass index (calculated as weight in kilograms divided by square of height in meters); ETS, environmental tobacco smoke; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF_{25%–75%}, forced expiratory flow at 25% to 75% of FVC; LCA, latent class analysis; PC₂₀, methacholine dose that caused a 20% reduction in FEV₁.

^aData are expressed as number (percentage) or mean (range).

^bComparison between participants in 4 atopy phenotypes and those excluded in the LCA.

^cComparison between participants including those in the 4 atopy phenotypes, controls, and those excluded in this study.

Table 2
Results of Latent Class Analysis

Variable	No. (%) of study participants					P value ^a
	Control	Cluster 1	Cluster 2	Cluster 3	Cluster 4	
Total	665 (51.9)	262 (20.5)	53 (4.1)	35 (2.7)	266 (20.8)	
Male	297/665 (44.7)	151/262 (57.6)	32/53 (60.4)	19/35 (54.3)	146/266 (54.9)	.001
BMI, normal	564/660 (85.5)	219/259 (84.6)	46/53 (86.8)	27/34 (79.4)	220/265 (83.0)	
Parental history of allergic diseases	226/656 (34.5)	94/259 (36.3)	33/53 (62.3)	15/34 (44.1)	114/263 (43.4)	<.001
Monthly income levels, \$US						
<3,000	486/659 (73.8)	167/254 (65.8)	33/53 (62.3)	26/34 (76.5)	181/262 (69.1)	.07
Maternal educational level						
High school or less	388/602 (64.5)	155/232 (66.8)	25/49 (51.0)	18/30 (60.0)	154/245 (62.9)	.312
Exposure to ETS, yes	284/611 (46.5)	110/227 (48.5)	16/47 (34.0)	14/33 (42.4)	109/241 (45.2)	.47
No. of sensitized allergens on skin prick tests						<.001
0	664/664 (100.0)	262/262 (100.0)	0/53 (0.0)	0/35 (0.0)	0/265 (0.0)	
1	0/664 (0.0)	0/262 (0.0)	0/53 (0.0)	25/35 (71.4)	88/265 (33.2)	
2	0/664 (0.0)	0/262 (0.0)	4/53 (7.6)	5/35 (14.3)	165/265 (62.3)	
≥3	0/664 (0.0)	0/262 (0.0)	49/53 (74.2)	5/35 (7.6)	12/265 (18.2)	
Total serum IgE level ≥100 IU/mL	210/660 (31.8)	135/262 (51.5)	51/53 (96.2)	22/35 (62.9)	196/263 (74.5)	<.001
Blood eosinophils ≥4%	139/661 (21.0)	87/262 (33.2)	44/53 (83.0)	12/35 (34.3)	158/264 (59.9)	<.001
Sensitization patterns						
Indoor allergens	0/665 (0.0)	0/262 (0.0)	53/53 (100.0)	0/35 (0.0)	266/266 (100.0)	<.001
Outdoor allergens	0/665 (0)	0/262 (0)	43/53 (81.1)	35/35 (100)	3/265 (1.1)	<.001
Food allergens	0/665 (0)	0/262 (0)	4/53 (7.6)	4/35 (11.4)	0/265 (0)	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by square of height in meters); ETS, environmental tobacco smoke.

^aCalculated using the χ^2 test or Fisher exact test, as appropriate.

indoor allergens at enrollment (Fig 2B). *D. pteronyssinus* (224 [84.5%]) and *D. farinae* (188 [70.9%]) were the most commonly sensitized indoor allergens at enrollment (Fig 1A). During the follow-up, participants in this cluster were also found to be

sensitized to outdoor allergens, including tree mix I (22 [8.3%]) and II (24 [9.0%]), mugwort (13 [4.9%]), and *Alternaria* (8 [3.0%]), with the most common sensitization being to cat epithelium (32 [12.0%]) and dog dander (48 [18.1%]).

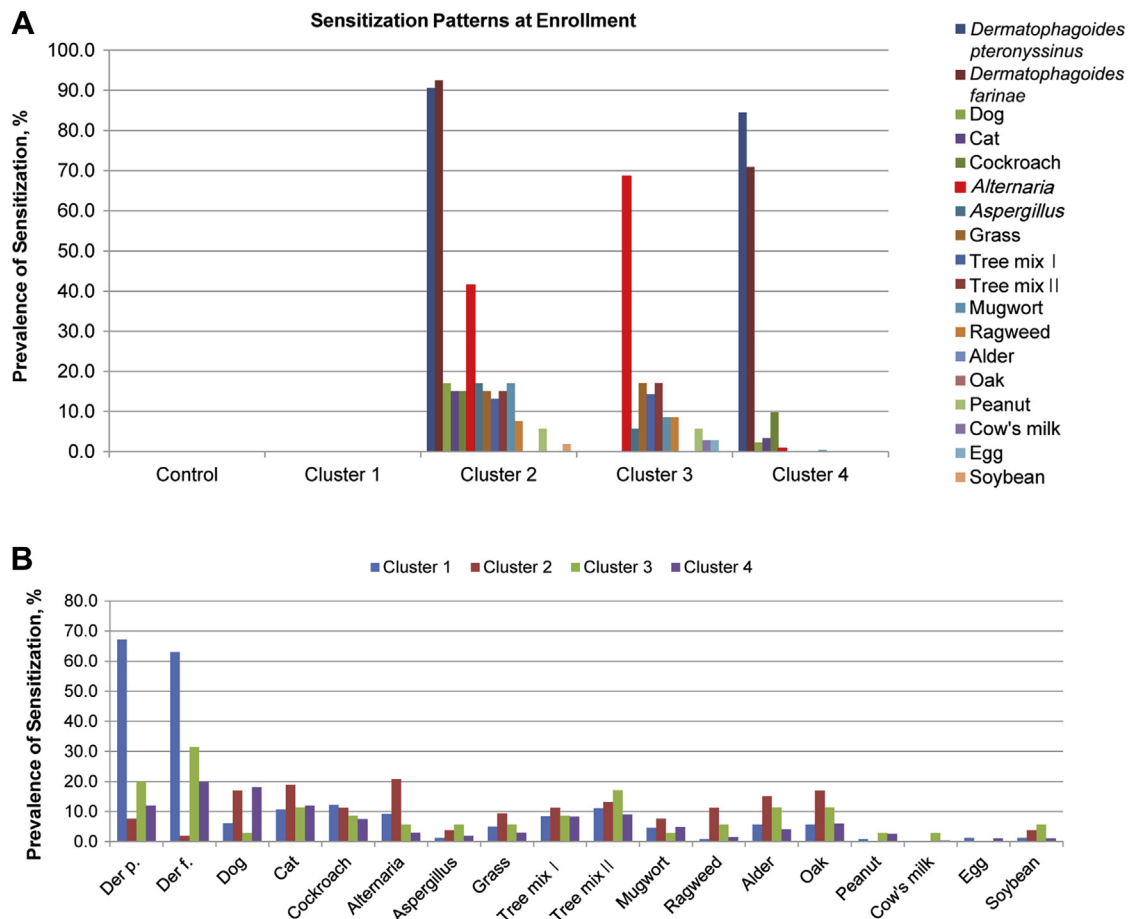


Figure 1. Characteristics of sensitization patterns according to atopy phenotype. A, Newly sensitized allergens during the prospective follow-up according to atopy phenotype. B, Sensitized allergens at enrollment.

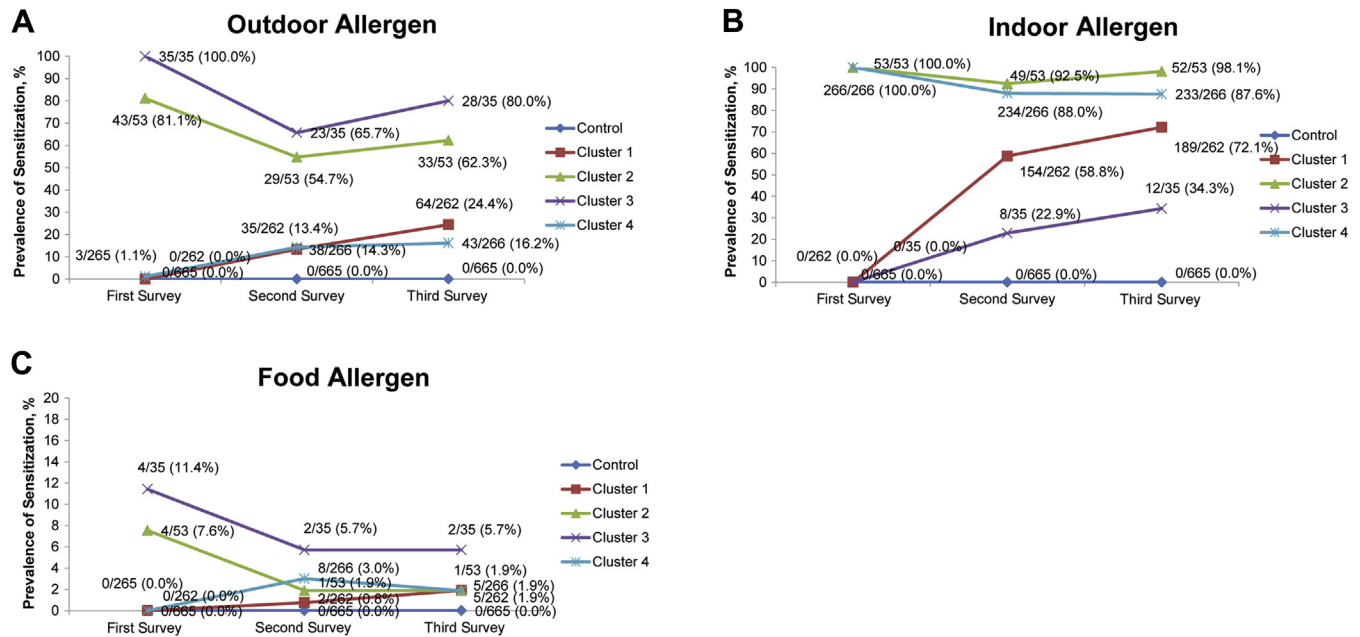


Figure 2. Sensitization patterns according to atopy phenotypes during the prospective follow-up. A, Sensitization to outdoor allergens. B, Sensitization to indoor allergens. C, Sensitization to food allergens.

Comorbidities With Each Atopy Phenotype

The prevalence of parental-reported, physician-diagnosed asthma in lifetime was highest in cluster 3 at enrollment (7 [20.6%] of 34) (Table 3). The frequency of asthma symptoms during the preceding 12 months were significantly increased in cluster 4 (adjusted aOR, 2.36; 95% CI, 1.56–3.57), when controls were considered as reference (eTable 1).

Clusters 2 and 4 had significantly higher BHR than the controls at enrollment (aOR, 4.30; 95% CI, 2.31–7.98; aOR, 2.19; 95% CI, 1.54–3.10, respectively) (eTable 1).

The prevalence of parental-reported, physician-diagnosed AR in lifetime was significantly increased in clusters 1, 2, and 4, with the highest prevalence observed in cluster 2 (controls as reference) (Table 3 and eTable 1). The prevalence of AR symptoms during the preceding 12 months was significantly increased in clusters 2, 3, and 4, with the highest prevalence observed in cluster 2 (aOR, 3.37; 95% CI, 1.75–6.51).

The prevalence of parental-reported, physician-diagnosed AD was significantly increased in clusters 1 and 3, when controls were considered as the reference. The prevalence of AD symptoms in the preceding 12 months was significantly increased in cluster 3 (aOR, 2.61; 95% CI, 1.22–5.58).

There was no significant association between any atopy cluster and parental-reported, physician-diagnosed FA.

Prognosis of Each Atopy Phenotype

The number of new cases of asthma during the prospective follow-up period was significantly increased in cluster 3 (aOR, 4.25; 95% CI, 1.46–12.36) (Fig 3A). The risk of new-onset BHR was significantly increased in cluster 3 (aOR, 5.03; 95% CI, 1.85–13.69), followed by cluster 4 (aOR, 3.75; 95% CI, 2.24–6.29), cluster 2 (aOR, 3.16; 95% CI, 1.11–9.05), and cluster 1 (aOR, 2.42; 95% CI, 1.42–4.12) (Fig 3B). On the other hand, the prevalence of new-onset AR was highest in cluster 2 (aOR, 7.21; 95% CI, 2.90–17.95), followed by cluster 4 (aOR, 2.37; 95% CI, 1.59–3.54) (Fig 3C). The risk of new cases of AD was significantly increased in cluster 4 (aOR, 1.68; 95% CI, 1.03–2.72) (Fig 3D).

The levels of FEV₁ were lowest in cluster 3 both at enrollment and the final survey (mean, 104.47%; 95% CI, 99.95%–108.99%; mean, 96.91%; 95% CI, 93.47%–100.36%, respectively) (Table 4); however, there were no statistical significances in these values. The levels of FEF_{25–75%} were decreased in cluster 3, compared with other clusters, at enrollment, although the difference was not significant (mean, 93.15%; 95% CI, 86.17%–100.13%) (Table 4).

Discussion

In the present study, the incidence of new-onset asthma and BHR was increased in school-age children sensitized to outdoor

Table 3
Prevalence of Combined Allergic Diseases at Enrollment According to Atopy Phenotype

Atopy phenotype	No. (%) of study participants					P value ^a
	Control	Cluster 1	Cluster 2	Cluster 3	Cluster 4	
Physician-diagnosed asthma, ever	46/635 (7.2)	24/243 (9.9)	7/49 (14.3)	7/34 (20.6)	51/252 (20.2)	<.001
Physician-diagnosed AR, ever	114/630 (18.1)	63/243 (25.9)	26/48 (54.2)	10/33 (30.3)	85/253 (33.6)	<.001
Physician-diagnosed AD, ever	177/624 (28.4)	90/242 (37.2)	19/49 (38.8)	15/33 (45.5)	87/253 (34.4)	.02
Physician-diagnosed FA, ever	32/628 (5.1)	11/244 (4.5)	4/48 (8.3)	2/33 (6.1)	14/250 (5.6)	.86
BHR (PC ₂₀ ≤ 8 mg/mL), ever	111/648 (17.1)	45/258 (17.4)	21/49 (42.9)	8/35 (22.9)	77/255 (30.2)	<.001

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; BHR, bronchial hyperresponsiveness; FA, food allergy; PC₂₀, methacholine dose that caused a 20% reduction in forced expiratory volume in 1 second.

^aCalculated using the χ^2 test or Fisher exact tests, as appropriate.

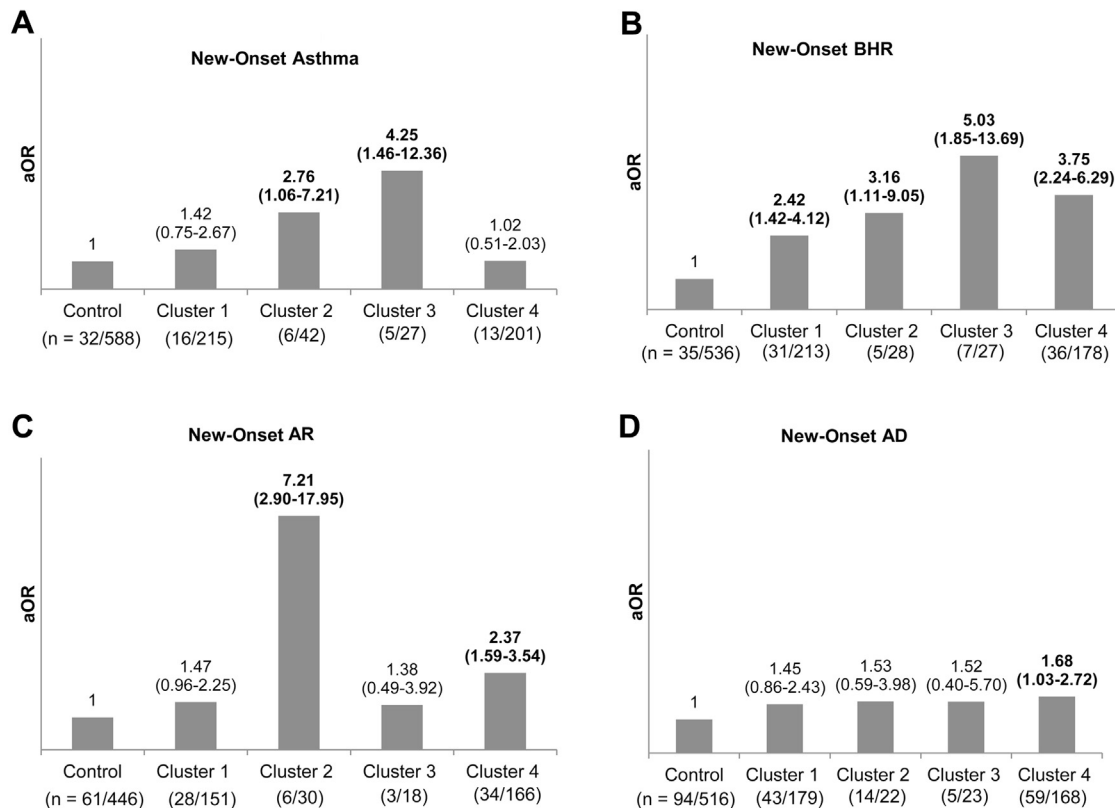


Figure 3. Prognosis of atopy phenotypes during the prospective follow-up. A, Association with new-onset asthma according to atopy phenotypes. B, Association with new-onset bronchial hyperresponsiveness (BHR) (methacholine dose that caused a 20% reduction in FEV₁ [PC₂₀] <8 mg/mL) according to atopy phenotype. C, Association with new-onset allergic rhinitis (AR) according to atopy phenotype. D, Association with new-onset atopic dermatitis (AD) according to atopy phenotype. BHR was defined as PC₂₀ of less than 8 mg/mL on methacholine provocation tests. Adjustment was performed for sex, age, body mass index, household income, and parental history of allergic diseases. aOR indicates adjusted odds ratio.

allergens, particularly to *Alternaria*, and with later-onset sensitization to indoor allergens and *Aspergillus*. This cluster had the lowest FEV₁ and FEV_{25–75} levels at the final survey, although there were no significant differences in the values. Early sensitization to indoor allergens increased the AR risk at enrollment and the new-onset AR during the follow-up period compared with early sensitization to outdoor allergens. The results of this study indicate that the types of sensitized allergen according to age are more important predictors of the progression of allergic diseases than atopy itself. This finding suggests that an understanding of the atopy phenotypes may facilitate the adoption of preventive strategies that can modify the progression of allergic diseases. Moreover, the present study identified an association between mold sensitization and an increased risk of asthma and BHR according to atopy phenotypes.

In previous studies of atopy phenotypes,^{6,12,13} high sensitization or multiple early sensitization was associated with an increased risk of later development of asthma in children. However, these previous studies did not consider the diverse sensitization patterns according to age, and some of these studies focused on a smaller number of allergens compared with that in the present study. Previous reports also have focused on the sensitization to pollens in young infants, an age at which the sensitization to pollen allergens is uncommon.¹² Moreover, some previous studies are limited in their assessment of the associations between new-onset asthma and/or the trajectories of lung function during the follow-up period.¹² In a recent high-risk birth cohort study,¹⁴ sensitization to multiple allergens were repetitively evaluated using both SPTs and specific IgE levels until the age of 13 years. However, the study had limitations in its assessment of the chronological changes in sensitizations in an individual, with regard to asthma and AD.

The strengths of the present study included its evaluation of the chronological changes within distinct atopy clusters and the assessment of more diverse outcomes and prognosis, in addition to asthma, BHR, and lung function, according to the atopy phenotypes. The present study also covered a more diverse allergen range than the previous studies.^{6,12} Furthermore, we repetitively performed objective assessments of asthma using spirometry and methacholine challenge tests at each survey, even though the follow-up duration was relatively short. Moreover, as our investigations were performed on a nationwide general population, our findings can be generalized to the school-age children.

Sensitization to mold at enrollment was highest in cluster 3 (26 [74.3%] of 35), characterized by an increased risk of new-onset asthma and BHR, followed by cluster 2 (29 [54.7%] of 53) and then cluster 4 (2 [0.8%] of 265). This finding is in agreement with those of previous studies, which indicated a positive association between sensitization to mold and asthma.²³ However, previous studies of atopy phenotypes did not describe an association between sensitization to mold and allergic diseases.^{6,12,13} In cross-sectional studies, sensitization to *Alternaria* was associated with the development of asthma, particularly a severe and persistent asthma with some debates.^{24–27} Thus, sensitization to *Alternaria* per se, with that to other outdoor allergens, and later sensitization to indoor allergens with *Aspergillus* may be associated with the development of asthma and BHR in later life. Early sensitization to *Alternaria* may play a role as a triggering factor for immune modulation; additional sensitization to mold allergens, even contained in house dust,¹ may increase the risk of asthma and BHR in later life. Previous studies have focused on the association between mold sensitization and an increased risk of allergic diseases, but there has

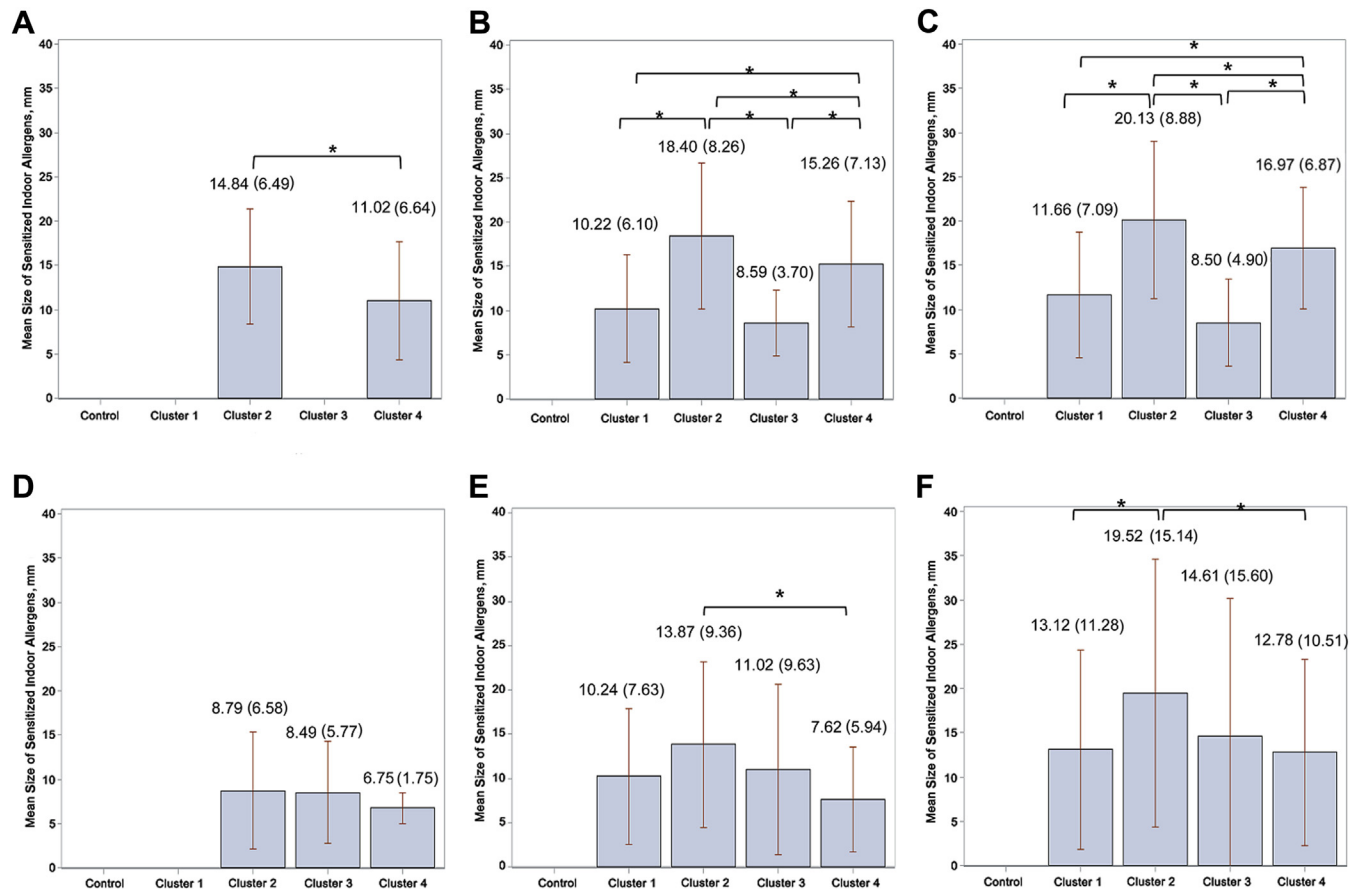


Figure 4. Mean (SD) wheal size of sensitized allergens. Mean wheal size of sensitized indoor allergens at enrollment (A), at the second survey (B), and at the final survey (C). Mean wheal size of sensitized outdoor allergens at enrollment (D), at the second survey (E), and at the final survey (F). * $P < .05$ using χ^2 test.

been a lack of attention on the role of species-specific sensitization to mold allergens.¹ Furthermore, although cross-reactivity among diverse mold allergens may affect the development and exacerbation of allergic diseases,^{1,2,28–31} the findings of the current study suggest different effects of sensitizations to species-specific mold allergens on the development of allergic diseases.

Sensitization to mold might be associated with the development of asthma and BHR through an immunologic or allergic effect.³² In contrast to the previous studies, which found an association between sensitization to *Alternaria* and AR,³³ our current findings indicate that sensitization to mold, including *Alternaria*, is more associated with asthma and BHR than AR. Outdoor allergens, including mold, have large particles that cannot access the lower airways. These particles damage the airway epithelium through a manner of complex interactions with the immune system, including NLRP3 inflammasome, which may contribute to the development of asthma and BHR over AR.^{34,35} Furthermore, fungal proteases can promote airway hyperresponsiveness through their infiltration of the bronchial submucosa, which results in the disruption of airway smooth muscle cells and extracellular matrix interactions.³⁶ Severe asthma with fungal sensitization has been introduced as a new phenotype, although its underlying mechanisms remain unclear.³⁷ Further studies on the pathogenesis of asthma and BHR in association with mold sensitization are needed to identify the interventions and develop personalized care.

In contrast, common sensitization to indoor allergens was more closely associated with an increased risk of AR compared with asthma and BHR in our study, despite previous studies reporting that such sensitization is strongly associated with asthma.³⁸

Although exposure to indoor aeroallergens in early life may not be associated with sensitization to indoor allergens,³⁹ the prevention of sensitization to indoor allergens might have protective effects on AR development. The mechanisms underlying the more positive association between sensitization to indoor allergens and AR, rather than asthma, remain unclear. Nevertheless, the effect of the sensitization to specific allergens on the development of allergic diseases may involve various factors, such as exposure duration, intensity of sensitization, and exposure to specific allergens.

Although the summed wheal diameters have also been associated with atopy,^{40,41} there were no significant differences in the summed wheal diameters across the atopy phenotypes with regard to indoor and outdoor allergens in our current study (Fig 4). Thus, the types of sensitized allergens, rather than summated wheal diameters, may be more important predictors of the prognosis in individuals with specific sensitization patterns. However, further studies are required to address this possibility.

This study had several limitations of note. First, the follow-up duration was relatively short. Given that sensitization to pollen is usually observed after 5 years of age,⁷ the present study enrolled children at a critical time before the potential sensitization. Second, some of the participants were lost to follow-up during the study period; nevertheless, the follow-up rate in the present study was still relatively high.²⁰ In addition, we used the results of SPTs to define atopy. Although one previous study has suggested that there is a poor association between SPTs and specific IgE levels before 6 years of age,⁴² we included children 7 years and older, and many of the previous studies on atopy phenotypes only considered

Table 4
Mean (95% CI) Total Serum IgE Levels, Blood Eosinophil Counts, and Lung Function According to Atopy Phenotype

Variable	At enrollment		Follow-up			
	Control		Cluster 1	Cluster 2	Cluster 3	Cluster 4
Total serum IgE, kU/L	139.9 (115.7–164.1) (n = 660)	3.0	295.8 ^a (209.2–382.4) (n = 262)	769.0 ^{a,b} (464.1–1021.0) (n = 53)	363.2 ^{a,c} (208.3–518.1) (n = 35)	517.7 ^{a,b,c} (429.8–605.6) (n = 263)
Blood eosinophils, %	(2.9–3.2) (n = 661)	3.7 ^a	(3.4–4.1) (n = 262)	(5.9–7.6) (n = 53)	(2.8–4.0) (n = 35)	(4.7–5.5) (n = 264)
FEV ₁ , % predicted	106.3 (105.3–107.3) (n = 658)	104.5	106.4 (104.7–108.0) (n = 258)	104.5 (100.7–108.4) (n = 52)	104.5 (100.0–109.0) (n = 34)	105.7 (104.0–107.4) (n = 261)
FVC, % predicted	100.0 (99.0–101.0) (n = 658)	91.4	100.8 (99.2–102.4) (n = 258)	99.1 (95.7–102.5) (n = 52)	98.4 (93.9–102.8) (n = 34)	100.0 (98.4–101.5) (n = 262)
FEV ₁ /FVC, % predicted	91.4 (91.0–91.8) (n = 661)	90.3 ^a	90.4 ^a (89.7–91.1) (n = 262)	90.1 (88.5–91.7) (n = 53)	91.1 (89.3–93.0) (n = 34)	90.3 ^a (89.6–91.0) (n = 264)
FEF _{25%–75%} , % predicted	100.3 (98.3–102.2) (n = 519)	99.5	98.9 (96.0–101.9) (n = 214)	99.5 (90.3–108.7) (n = 36)	93.2 (86.2–100.1) (n = 27)	97.6 (94.5–100.6) (n = 211)

Abbreviations: FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF_{25%–75%}, forced expiratory flow at 25% to 75% of FVC.^ap < .05, compared with control group.^bp < .05, compared with cluster 1.^cp < .05, compared with cluster 2.^dp < .05, compared with cluster 3.

SPTs and/or specific IgE.^{12,13} In addition, although the lack of reproducibility of skin tests among the testers may represent a limitation of the study, the testers were trained using standardized protocols⁴³ and had considerable experience with SPTs. We did not assess the amount of exposed allergens in the environment. Therefore, our study was limited in its association of exposure degree with sensitization.⁴⁴ Because our current investigation was questionnaire based, asthma severity was not assessed and there might be a limitation in the accurate diagnosis of allergic diseases. However, our findings are meaningful in that they were obtained from a general population, in contrast to other hospital or patient-based studies.

In conclusion, sensitization to outdoor allergens, especially mold, and later sensitization to indoor allergens in school-age children is associated with an increased risk of new-onset asthma and BHR. Early sensitization to indoor allergens is associated with an increased risk of AR. The sequential differences in sensitization to indoor or outdoor allergens, particularly mold allergens, might differently affect the development of allergic diseases. The identification of atopy phenotypes seems to facilitate the prediction of the development of allergic diseases in school-age children and suggests the need for the prevention of additional sensitization to modify the progression of allergic diseases.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2017.02.008>.

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eTable 1

Comorbidities of Allergic Diseases According to Atopy Phenotypes at Enrollment

	Variables	Control	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Asthma	Diagnosis by physician	Ref	1.331 (0.79–2.26)	1.61 (0.67–3.86)	3.28 (1.32–8.14)	2.91 (1.87–4.52)
	Symptoms in the last 12 months	Ref	1.022 (0.62–1.70)	2.14 (0.99–4.57)	1.24 (0.42–3.73)	2.36 (1.56–3.57)
	Treatment in the last 12 months	Ref	1.669 (0.74–3.79)	3.82 (1.30–11.25)	1.26 (0.16–9.91)	5.26 (2.76–10.00)
	Current asthma	Ref	1.073 (0.48–2.41)	3.21 (1.19–8.64)	2.92 (0.81–10.55)	4.24 (2.37–7.60)
AR	Diagnosis by physician	Ref	1.474 (1.01–2.14)	4.03 (2.12–7.65)	1.65 (0.72–3.81)	2.11 (1.49–2.98)
	Symptoms in the last 12 months	Ref	1.025 (0.75–1.41)	3.37 (1.75–6.51)	2.24 (1.04–4.80)	1.64 (1.21–2.22)
	Treatment in the last 12 months	Ref	1.023 (0.68–1.54)	2.34 (1.22–4.48)	1.28 (0.52–3.15)	1.92 (1.34–2.74)
	Current AR	Ref	1.129 (0.82–1.55)	4.39 (2.16–8.92)	2.40 (1.10–5.22)	1.69 (1.25–2.29)
AD	Diagnosis by physician	Ref	1.415 (1.02–1.96)	1.33 (0.72–2.47)	2.33 (1.10–4.93)	1.30 (0.94–1.79)
	Symptoms in the last 12 months	Ref	1.16 (0.81–1.67)	0.59 (0.27–1.30)	2.61 (1.22–5.58)	1.33 (0.94–1.88)
	Treatment in the last 12 months	Ref	1.357 (0.87–2.13)	0.87 (0.35–2.15)	1.78 (0.69–4.60)	1.61 (1.07–2.45)
	Current AD	Ref	1.294 (0.94–1.78)	1.10 (0.59–2.03)	1.91 (0.91–4.04)	1.29 (0.94–1.77)
FA	Diagnosis by physician	Ref	0.931 (0.46–1.90)	1.64 (0.54–4.95)	0.62 (0.08–4.75)	1.14 (0.59–2.19)
	Symptoms in the last 12 months	Ref	0.974 (0.49–1.94)	2.25 (0.88–5.79)	1.20 (0.27–5.32)	1.30 (0.71–2.41)
	Treatment in the last 12 months	Ref	1.188 (0.47–2.98)	5.16 (1.84–14.31)	1.25 (0.16–9.86)	1.64 (0.72–3.73)
	Current FA	Ref	1.081 (0.61–1.92)	2.09 (0.86–4.93)	0.88 (0.20–3.83)	1.32 (0.77–2.25)
BHR	PC20 ≤ 8 mg/mL	Ref	1.008 (0.68–1.51)	4.30 (2.31–7.98)	1.68 (0.73–3.87)	2.19 (1.54–3.10)

AD, atopic dermatitis; AR, allergic rhinitis; BHR, bronchial hyperresponsiveness; FA, food allergy; Ref, Reference.

Values in boldface indicate statistical significance.