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Heterogeneity of Childhood Asthma in Korea: Cluster Analysis of the Korean Childhood Asthma Study Cohort

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ABSTRACT

Purpose: Asthma is a heterogeneous airway disease occurring in children, and it has various clinical phenotypes. A clear differentiation of the clinical phenotypes can provide better asthma management and prediction of asthma prognosis. Little is currently known about asthma phenotypes in Korean children. This study was designed to identify asthma phenotypes in school-aged Korean children.
Methods: This study enrolled 674 children with physician-diagnosed asthma from the Korean childhood Asthma Study (KAS) cohort. The physicians verified the relevant histories of asthma and comorbid diseases, as well as airway lability and hyper-responsiveness from

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Disclosure

the results of pulmonary function tests and bronchial provocation tests. Questionnaires regarding the participants' baseline characteristics, their environment and self-rating of asthma control were collected at the time of enrollment. Laboratory tests were performed to assess allergy and airway inflammation. Children with asthma were classified by hierarchical cluster analysis.

Results: Of the 674 patients enrolled from the KAS cohort, 447 were included in the cluster analysis. Cluster analysis of these 447 children revealed 4 asthma phenotypes: cluster 1 (n = 216, 48.3%) which was characterized by male-dominant atopic asthma; cluster 2 (n = 79, 17.7%) which was characterized by early-onset atopic asthma with atopic dermatitis; cluster 3 (n = 47, 10.5%) which was characterized by puberty-onset, female-dominant atopic asthma with the low lung function; and cluster 4 (n = 105, 23.5%) which was characterized by early-onset, non-atopic dominant asthma.

Conclusions: The asthma phenotypes among Korean children can be classified into 4 distinct clusters. Long-term follow-up with these phenotypes will be needed to define their prognosis and response to treatment.

Keywords: Asthma; childhood; phenotype; cluster analysis

INTRODUCTION

Asthma is a heterogeneous airway disease and occurs in various clinical phenotypes. These phenotypes differ in physiological and biochemical characteristics, and the various endotypes of asthma are characterized by distinct functional and/or pathophysiological mechanisms.[1-4] A clear differentiation of these clinical phenotypes and endotypes is necessary to improve asthma management, thereby improving and personalizing asthma treatment.[5-7] Unsupervised cluster analysis is a statistical method frequently utilized in clinical studies to group participants with similar characteristics. This has also been used to identify discrete asthma phenotypes in several cohorts of patients varying in age, demographic characteristics, and underlying asthma severity.[8]

Childhood asthma phenotypes have been classified in children with severe asthma.[9-13] These studies suggested the differences in severe asthma phenotypes in children stratified by onset age, atopy, sex, treatment, and lung function, suggesting that asthma is clinically heterogeneous and that new approaches are required for the classification of asthma severity. However, asthma phenotype clustering within general population-based studies is insufficient. Furthermore, because most of the previous studies have assessed patients in North America and Europe, their results may not be applicable to Asian populations, due to ethnic differences based on genetic effects.[14] Analysis of the progression of childhood asthma over time and comparisons of asthma exacerbation in clusters require prospective studies to identify participants' asthma phenotypes and to suggest appropriate intervention to prevent progression to adult asthma.

Few studies to date have assessed asthma phenotypes in Korean children.[15] The purpose of this study aimed to determine asthma phenotypes in school-aged Korean children by cluster analysis of the Korean childhood Asthma Study (KAS) cohort.

MATERIALS AND METHODS

Overall design of the KAS cohort

The KAS is a nationwide, 19-center prospective cohort study of 1,000 participants aged 5 to 15 years who were diagnosed with childhood asthma by pediatric allergists and pulmonologists. Children who experienced typical symptoms of asthma (*i.e.*, wheezing, dyspnea, and chronic cough) within the recent 12 month period were included if they showed either an elevated bronchodilator response (BDR) (*i.e.*, a ≥ 12% increase of forced expiratory volume in 1 second [FEV_1] 15 minutes after inhaling 200 mcg of salbutamol) or bronchial hyper-responsiveness (BHR) (*i.e.*, a < 16 mg/mL dose of provocative methacholine concentration causing a 20% reduction in FEV_1 [PC20] or a < 635 mg dose of provocative mannitol weight causing a 15% reduction in FEV_1 [PD15]).[16,17] Methacholine provocation test was conducted on 498 (73.9%) participants, and mannitol provocation test was conducted on 41 (6.1%) participants. Children who exhibited interstitial lung diseases or pulmonary neoplasms were excluded. Researchers collected the participants' baseline characteristics using a set of questionnaires assessing the frequency of asthma symptoms within 3 months, and history of health-care use (≥ 1 hospitalization or emergency department [ED] visit). Pulmonary function data, including BHR, blood samples and skin prick test results for allergies, as well as additional potential variables were also evaluated. Predicted values of spirometry were based on global lung initiative reference equations.[18] Evaluation of asthma severity was based on the physician's subjective clinical assessment using the National Asthma Education and Prevention Program (NAEPP) guidelines.[19] No treatment intervention was involved. All participants continued to receive asthma medication as directed by the NAEPP guidelines.[11] Participants' responses to treatment, including levels of asthma control and episodes of exacerbation, lung function, and changes in the body's physical and environmental factors, were evaluated at least once every 6 months during regular visits. BHR and atopy were measured every 3 years during the study period. Changes in all the above variables were compared among clusters at baseline and every 3 years during the study period. The follow-up duration of this cohort is planned as every 6 months for the next 3 to 5 years. The study methods have been detailed elsewhere.[20] We included 674 of KAS children in our study from September, 2016 to August, 2018. The study was approved by the Institutional Review Boards (IRBs) of Asan Medical Center (IRB No. 2016-0914), Seoul National University Hospital (IRB No. 1607-165-779), Pusan National University Yangsan Hospital (IRB No. 05-2016-121), Inha University Hospital (IRB No. 2016-07-016-008), Seoul National University Bundang Hospital (IRB No. 10-2017-036), Chonnam National University Hospital (IRB No. 2017-201), Korea University Anam Hospital (IRB No. 2015 AN 0310), Soonchunhyang University Hospital in Seoul (IRB No. 2017-01-011-002), Bucheon St. Mary's Hospital (IRB No. HC16SNMI0056), Sungkyunkwan University Samsung Changwon Hospital (IRB No. 2017-02-006-001), Kangdong Sacred Heart Hospital (IRB No. 2016-12-007-001), The Catholic University of Korea, Uijeongbu St. Mary's Hospital (IRB No. UC16ONMI0113), Chungbuk National University Hospital (IRB No. 2016-09-003), Dankook University Hospital (IRB No. 2017-02-013), Korea University Guro Hospital (IRB No. 2016GR0336), Inje University Seoul Paik Hospital (IRB No. 2016-314), CHA Gangnam Medical Center (IRB No. GCI-16-37), National Health Insurance Service Ilsan Hospital (IRB No. NHIMC 2017-02-008), and Soonchunhyang University School of Medicine in Bucheon (IRB No 2016-08-007-009). Written informed consent was obtained from all parents and the guardians of all patients after a detailed explanation of the study.

Variable selection

Cluster-defining variables were selected from the KAS baseline variables based on clinical meaningfulness and minimal missing data. From an initial list of clinical variables, 12 were

selected as representative of each child's objective factors associated with increased asthma burdens as inputs for the clustering algorithm. The final set of variables used in the cluster analyses was as follows: 1) sex; 2) age; 3) current diagnosis of allergic rhinitis (AR) (exhibiting AR symptoms within the last 12 months and a diagnosis of AR by clinicians at baseline); 4) current diagnosis of atopic dermatitis (AD) (exhibiting AD symptoms within the last 12 months and a diagnosis of AD by clinicians at baseline); 5) lifetime history of AD diagnosis; 6) history of acute bronchiolitis; 7) puberty stage[21]; 8) age at asthma onset; 9) PC20 from the methacholine challenge test performed according to the American Thoracic Society guidelines[16]; 10) atopy defined as a positive response to at least 1 allergen on skin prick tests; 11) baseline predicted FEV_1 (%); and 12) frequency of asthma symptoms.

The outcome variables evaluated included age at the onset of asthma symptoms, asthma severity, methacholine PC20 (mg/mL), body mass index (BMI, kg/m^2), puberty stage based on Tanner stage,[22] use of inhaled corticosteroid (ICS), use of controller medications, any emergency department visit or hospitalization due to asthma exacerbation during the previous 12 months, any use of systemic corticosteroid burst due to asthma exacerbation during the previous 12 months, and pre- and postbronchodilator lung function at baseline and 6 months.[23]

Cluster and statistical analyses

Cluster analysis was performed at baseline as cross-sectional variables using a hierarchical clustering algorithm with the Ward minimum variance method, which has the advantage of minimizing the total within-cluster variance.[24] Stepwise discriminant analysis of the cluster variables was performed to determine the strongest predictors of cluster assignment (**Supplementary Table S1**).[25] Missing values were excluded from the analysis without missing value imputation. Differences among clusters were compared by ANOVA for continuous variables and χ^2 tests for categorical variables, with Bonferroni correction adjustment for multiple comparisons. Variables with significant ($P < 0.05$) differences among clusters were considered as candidate distinguishing features. Data were analyzed using commercially available statistical software, the Statistical Analysis System (SAS) version 9.4 (SAS Inc., Cary, NC, USA).

RESULTS

The baseline characteristics of all 674 patients are presented in **Table 1**. Participants who missed one or more of the cluster variables were excluded. Among these 674 patients, 447 were available for cluster analysis (**Table 1**, **Supplementary Table S2**). There were significant differences in puberty rate, atopy rate, and baseline FEV_1 (% predicted) between 447 participants included in the cluster analysis and 227 participants excluded from the analysis due to missing values in any of the variables. Other variables showed no statistically significant differences (**Table 2**).

Cluster analysis

The hierarchical clustering algorithm approach identified 4 clusters distinguished by age, sex, current diagnosis of AR, current diagnosis of AD, lifetime diagnosis of AD, history of acute bronchiolitis, puberty stage, age at asthma onset, methacholine PC20, atopy, baseline predicted FEV_1 (%), and frequency of asthma symptoms. Clusters also differed by medication use and other healthcare (**Table 3**), and lung function (**Table 4**).

Table 1. Baseline characteristics of study participants in total and according to cluster analysis

Characteristics	KAS in total (n = 674)
Age (yr)	9.0 ± 2.6
Male	445/674 (66.0)
Current AR diagnosis	530/669 (79.2)
Current AD diagnosis	146/670 (21.8)
Lifetime history of AD	261/662 (39.4)
History of acute bronchiolitis	225/651 (34.6)
Puberty	
I	481/659 (73.0)
II	105/659 (15.9)
III	40/659 (6.1)
IV	22/659 (3.3)
V	11/659 (1.7)
Age at asthma symptom onset (yr)	
< 3	109/659 (16.5)
≥ 3, < 6	223/659 (33.8)
≥ 6, < 9	181/659 (27.5)
≥ 9, < 12	103/659 (15.6)
≥ 12	43/659 (6.5)
Asthma severity	
Mild intermittent	251/667 (37.6)
Mild persistent	260/667 (39.0)
Moderate persistent	152/667 (22.8)
Severe persistent	4/667 (0.6)
Frequency of asthma symptoms	
None	154/644 (23.9)
< 1/month	197/644 (30.6)
≥ 1/month, < week	148/644 (23.0)
≥ 1/week, < 2/week	59/644 (9.2)
≥ 2/week, < 1/day	53/644 (8.2)
≥ 1/day	33/644 (5.1)
Methacholine (PC20, mg/mL), mean (range)	1.9 (0.5–8.0)
Atopy (≥ 1 on skin prick test)	501/674 (74.3)
Baseline FEV_1 (% predicted)	90.4 ± 16.2
Bronchodilator response (%)	6.6 ± 9.0

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.
$P < 0.05$ by χ^2 tests.
KAS, Korean childhood Asthma Study; AR, allergic rhinitis; AD, atopic dermatitis; PC20, provocative methacholine concentration causing a 20% reduction in FEV_1; FEV_1, forced expiratory volume in 1 second.

Cluster 1

Cluster 1 compromised 216 (156 [72.2%], boys; 60 [27.8%], girls) children (45.8%). Cluster 1 was characterized by male-dominant atopic asthma. Their mean age was 8.8 ± 2.1 years. Additionally, 215 (99.5%) children also had current AR, and 209 (96.8%) were positive on skin prick tests, with a geometric mean IgE concentration of 347.2 kU/L (**Table 2**). Of these 216 children, 86 (40.0%) and 87 (40.5%) had mild intermittent type and mild persistent type asthma, respectively. The frequency of asthma symptoms was none or less than once per month in 128 (59.3%) children, and 156 (72.0%) used controller medication (**Table 3**). Despite having BHR to methacholine (PC20, 2.46 mg/mL; range of one standard deviation [SD], 0.58–10.49 mg/mL), these children had relatively preserved pulmonary function, with a predicted forced volume vital capacity (FVC) 100.6 ± 13.1%, a predicted FEV_1 94.3 ± 14.3%, a FEV_1/FVC 93.8 ± 8.9, and a predicted maximal mid-expiratory flow (MMEF) 82.4 ± 26.1%. The average BDR was 7.1 ± 10.0% (**Table 4**).

Table 2. Demographic characteristics of traits across clusters

Characteristics	Cluster 1	Cluster 2	Cluster 3	Cluster 4	P value	Bonferroni
Age	8.8 ± 2.1	8.9 ± 2.2	13.2 ± 1.8	7.5 ± 2.0	< 0.0001	3 vs. 2, 1, 4
Male	156 (72.2)	56 (70.9)	10 (21.3)	64 (61.0)	< 0.0001	1, 2 vs. 4
Asthma history					< 0.0001	1, 2, 4 vs. 3
Age of asthma onset (yr)						
< 3	35 (16.2)	18 (22.8)	0 (0)	22 (21.0)		
≥ 3, < 6	69 (31.9)	33 (41.8)	6 (12.8)	44 (41.9)		
≥ 6, < 9	76 (35.2)	22 (27.9)	6 (12.8)	23 (21.9)		
≥ 9, < 12	33 (15.3)	5 (6.3)	14 (29.8)	14 (13.3)		
≥ 12	3 (1.4)	1 (1.3)	21 (44.7)	2 (1.9)		
History of acute bronchiolitis	83 (38.4)	29 (36.7)	4 (8.5)	31 (29.5)	0.001	1, 2, 4 vs. 3
Atopic features						
Current AR diagnosis	215 (99.5)	68 (86.1)	37 (78.7)	38 (36.2)	< 0.0001	1, 2 vs. 3
Current AD diagnosis	5 (2.3)	78 (98.7)	11 (23.4)	0 (0.0)	< 0.0001	2, 3 vs. 4
Lifetime history of AD diagnosis	57 (26.4)	78 (98.7)	21 (44.7)	15 (14.3)	< 0.0001	1 vs. 2, 3, 4
Positive skin test response, atopy (%)	209 (96.8)	66 (83.5)	42 (89.4)	33 (31.4)	< 0.0001	2, 3 vs. 4
Total serum IgE levels, kU/L	347.2 (115.6–1,043.2)	415.7 (129.0–1,339.4)	347.2 (64.7–1,863.1)	149.9 (38.9–578.3)	< 0.0001	1 vs. 2, 3
Anthropomorphic features						
Tanner stage					< 0.0001	2, 4 vs. 3
I	178 (82.4)	68 (86.1)	2 (4.3)	95 (90.5)		
II	30 (13.9)	9 (11.4)	11 (23.4)	9 (8.6)		
III	6 (2.8)	2 (2.5)	11 (23.4)	1 (1.0)		
IV	2 (0.9)	0 (0.0)	16 (34.04)	0 (0.0)		
V	0 (0.0)	0 (0.0)	7 (14.89)	0 (0.0)		
BMI (kg/m²)	18.7 ± 3.6	18.3 ± 3.6	20.7 ± 3.9	17.8 ± 3.0	< 0.0001	3 vs. 1, 2, 4

All P values for the multiple comparisons were < 0.008 (the adjusted P value based on the Bonferroni correction). Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.
AR, allergic rhinitis; AD, atopic dermatitis; IgE, immunoglobulin E; BMI, body mass index.

Table 3. Asthma severity, medication, and healthcare use across clusters

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	P value	Bonferroni
Asthma severity					0.0002	2 vs. 3, 4
Mild intermittent	86 (40.0)	47 (59.5)	11 (23.4)	30 (28.6)		
Mild persistent	87 (40.5)	17 (21.5)	20 (42.6)	54 (51.4)		
Moderate persistent	40 (18.6)	15 (19.0)	16 (34.0)	21 (20.0)		
Severe persistent	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)		
Frequency of asthma symptoms					0.024	1, 2, 4 vs. 3
None	59 (27.3)	19 (24.1)	8 (17.0)	26 (24.8)		
< 1/month	69 (31.9)	26 (32.9)	14 (29.8)	32 (30.5)		
≥ 1/month, < 1/week	45 (20.8)	23 (29.1)	5 (10.6)	29 (27.6)		
≥ 1/week, < 2/week	20 (9.3)	2 (2.5)	8 (17.0)	7 (6.7)		
≥ 2/week, < 1/day	18 (8.3)	5 (6.3)	6 (12.8)	6 (5.7)		
≥ 1/day	5 (2.3)	4 (5.1)	6 (12.8)	5 (4.8)		
Use of ICS					0.006	2 vs. 3
Low-dose ICS	107 (50.5)	29 (37.2)	30 (63.8)	56 (53.9)		
Medium-dose ICS	30 (14.2)	8 (10.3)	11 (23.4)	13 (12.5)		
High-dose ICS	1 (0.5)	0 (0.0)	0 (0.0)	1 (1.0)		
Controller medications					0.003	2 vs. 3, 4
None	60 (28.0)	34 (43.0)	5 (10.6)	26 (25.0)		
Montelukast only	16 (7.5)	5 (6.3)	2 (4.3)	9 (8.7)		
ICS only	49 (22.9)	24 (30.4)	10 (21.3)	21 (20.2)		
ICS+montelukast	21 (9.8)	4 (5.1)	5 (10.6)	16 (15.4)		
ICS+LABA	20 (9.4)	4 (5.1)	7 (14.9)	12 (11.5)		
ICS+LABA+montelukast	48 (22.4)	8 (10.1)	18 (38.3)	20 (19.2)		
At least one systemic corticosteroid	70/214 (32.7)	22/77 (28.6)	11/45 (24.4)	33/104 (31.7)	0.697	
Healthcare use					0.447	
None	170 (78.7)	63 (79.8)	35 (74.5)	75 (71.4)		
≥ 1 hospitalization or ED visit	46 (21.3)	16 (20.3)	12 (25.5)	30 (28.6)		

All P values for the multiple comparisons were < 0.008 (the adjusted P value based on the Bonferroni correction). Data are presented as number (%).
ICS, inhaled corticosteroid; LABA, long acting beta-2 agonist; ED, emergency department.

Table 4. Pulmonary function variables across clusters

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	P value	Bonferroni
Prebronchodilator pulmonary function						
FEV$_1$ (% predicted)	94.3 ± 14.3	94.0 ± 13.0	84.6 ± 15.9*	95.9 ± 11.6	0.001	4, 1, 2 vs. 3
FVC (% predicted)	100.6 ± 13.1	99.0 ± 12.2	90.3 ± 13.7*	101.3 ± 12.0	< 0.0001	4, 1, 2 vs. 3
FEV$_1$/FVC	93.8 ± 8.9	95.1 ± 8.0	94.3 ± 17.6	95.0 ± 9.4	0.456	
MMEF (% predicted)	82.4 ± 26.1	86.1 ± 24.1	75.8 ± 28.3	84.2 ± 21.4	0.304	
Postbronchodilator pulmonary function						
FEV$_1$ (% predicted)	98.9 ± 14.5	99.0 ± 14.4	90.2 ± 16.0*	101.0 ± 12.7	0.007	4 vs. 3
FVC (% predicted)	100.6 ± 13.2	99.0 ± 13.5	93.9 ± 11.7	102.9 ± 12.7	0.015	
MMEF (% predicted)	104.2 ± 32.2	109.0 ± 30.7	90.2 ± 33.2	107.9 ± 28.0	0.090	
Airway responsiveness						
Methacholine PC20	2.5 (0.6–10.5)	1.8 (0.6–5.5)	2.1 (0.5–8.2)	1.4 (0.4–5.2)	0.004	1 vs. 4
Bronchodilator response (%)	7.1 ± 10.0	4.1 ± 5.7	10.6 ± 9.9	5.6 ± 7.9	0.011	3 vs. 2

All P values for the multiple comparisons were < 0.008 (the adjusted P value based on the Bonferroni correction). Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.
FEV$_1$, forced expiratory volume in 1 second; FVC, forced volume vital capacity; MMEF, maximal mid-expiratory flow; PC20, provocative methacholine concentration causing a 20% reduction in FEV$_1$.

Cluster 2

Cluster 2 comprised 79 children (36.6%) (mean age, 8.9 ± 2.1 years) and was characterized as having early-onset atopic asthma with AD. This group had an earlier onset of asthma symptoms with 78 (98.7%) having a significant rate of AD, and 66 (83.5%) having atopy. Because most patients in this cluster experienced asthma onset before the age of 6 years, and most had current AD (n = 78, 98.7%), lifetime history of AD (n = 78, 98.7%), and current AR (n = 68, 86.1%), it is highly likely that the disease in this group is equivalent to atopic march. Children in this group also had a high rate of atopy (n = 66, 83.5%), with a geometric mean IgE concentration of 415.7 kU/L (range of one SD, 129.0–1,339.4 kU/L) (**Table 2**). This cluster had the least severe type of asthma, with 47 (59.5%) having the mild intermittent type and 34 (43.0%) children did not use controller medications. The proportion of these patients requiring hospitalization or ED visit was the lowest among the 4 clusters, although the differences were not statistically significant among the clusters (**Table 3**). Their pulmonary function, including FEV$_1$, FVC, MMEF, and post-BDRs, was similar to that observed in cluster 1. The geometric mean of BHR to methacholine PC20 was 1.80 mg/mL (range of one SD, 0.6–5.5 mg/mL) and BDR was 4.1 ± 5.7% (**Table 4**).

Cluster 3

Cluster 3 was the smallest one, comprising 47 (37 [78.7%], girls; 10 [21.3%], boys) children (10.0%). Children in this cluster, termed puberty-onset, female-dominant atopic asthma, were older than those in the other clusters (mean age 13.2 ± 1.8 years). Moreover, this cluster was differentiated by late symptom onset, with 21 children (44.7%) experiencing symptom onset at age ≥ 12 years, and 45 (95.7%) having entered puberty (Tanner stages II–V). Forty-two children (89.4%) in this group had atopic features, with a geometric mean IgE concentration of 347.2 kU/L (range of one SD, 64.7–1863.1 kU/L). This cluster showed the highest BMI (kg/m^2) among the 4 cluster, but the value was within normal ranges (**Table 2**). Sixteen children (34.0%) had moderate persistent asthma, with this group containing the most severe cases, with the highest frequency of asthma symptoms, use of controller medications (n = 42, 89.4%), and proportion who used combination treatments (n = 30, 63.8%) among the 4 clusters. Twelve children in this group (25.5%) experienced asthma symptoms more than twice weekly or more than once daily (**Table 3**). This cluster was distinguished by the lowest pulmonary function, including the lowest predicted FEV$_1$ (84.6 ± 15.9%) and FVC (90.3 ± 13.7%), although both were within normal ranges. This group also showed the lowest postbronchodilator pulmonary function, including a predicted FEV$_1$ and FVC values of 90.2 ± 16.0% and 93.9 ± 11.7%, respectively. The

geometric mean of BHR to methacholine PC20 was 2.1 mg/mL (range of 1 SD, 0.5–8.2 mg/mL) and the mean of BDR was the highest among 4 groups (10.6 ± 9.9%) (**Table 4**).

Cluster 4

Cluster 4 comprised 105 children (22.2%), termed the early-onset, non-atopic dominant asthma group. Their mean age was 7.5 ± 2.0 years, and most children in this group first experienced asthma symptoms at the preschool age (n = 66, 62.9%). None of the patients in this cluster had a history of current AD, with this cluster having the lowest prevalence of AR (n = 38, 36.2%) and skin prick test reactivity (n = 33, 31.4%) among the 4 clusters. Their geometric mean IgE concentration was 149.9 kU/L (range of one SD, 38.9–579.3 KU/L), which was lower than those of the 3 other clusters (**Table 2**). The asthma symptom frequency in this group was similar to that in clusters 1 and 2, with 11 (10.5%) children experiencing asthma symptoms more than twice weekly. The proportion of patients in this cluster using non-controller medications (n = 26, 25.0%) was similar to that in cluster 1, and the proportion using controller medication (n = 56, 53.9%) was higher than that in cluster 2, with most of these patients using low-dose ICS (**Table 3**). Despite having BHR to methacholine (PC20, 1.40 mg/mL; range of 1 SD, 0.4–5.2 mg/mL) with BDR 5.6 ± 7.9%, their pulmonary function was preserved, with the highest predicted FEV_1 (95.9 ± 11.6%) (**Table 4**). The frequency of hospitalization or ED visit at least once during the previous year was similar in all 4 clusters (**Table 3**).

Major determinants of cluster assignment

Seven variables, including current diagnosis of AD ($P < 0.001$), puberty ($P < 0.001$), positive skin test response (atopy, $P < 0.001$), current diagnosis of AR ($P < 0.001$), age at asthma onset ($P < 0.001$), baseline % predicted FEV_1 ($P < 0.001$), and sex ($P < 0.001$), were identified as the strongest determinants of cluster assignment in this study (Wilks λ = 0.025; F = 157.18; $P < 0.0001$). These 7 variables resulted in the correct classification of 93.1% of the original participants (**Table 2**, **Fig. 1**).
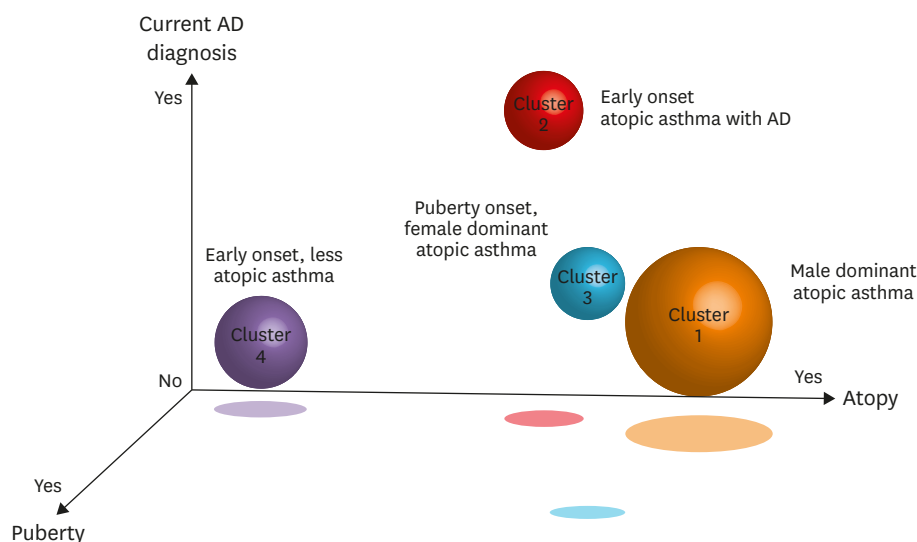

Figure. Distribution of the four clusters around the main variables of the three axes, current diagnosis of atopic dermatitis (AD), puberty, and atopy. Cluster 1, male-dominant atopic asthma; cluster 2, early onset atopic asthma with AD; cluster 3, puberty-onset, female-dominant atopic asthma; cluster 4, early onset, non-atopic dominant asthma.

Table 5. Pulmonary function variables after 6 months across clusters

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	P value	Bonferroni
Prebronchodilator pulmonary function						
FEV$_1$ (% predicted)	96.5 ± 12.5	95.8 ± 10.1	91.4 ± 7.9	96.9 ± 13.5	0.186	
FVC (% predicted)	101.0 ± 10.7	100.3 ± 9.8	93.8 ± 8.6	103.4 ± 12.1	0.008	4, 1 vs. 3
FEV$_1$/FVC	95.9 ± 11.8	95.8 ± 7.8	97.8 ± 7.6	93.7 ± 7.4	0.263	
MMEF (% predicted)	87.4 ± 28.9	84.9 ± 21.7	93.7 ± 22.3	85.3 ± 23.7	0.558	
Postbronchodilator pulmonary function						
FEV$_1$ (% predicted)	101.1 ± 11.1	100.6 ± 9.9	92.8 ± 8.3	101.3 ± 15.2	0.122	
FVC (% predicted)	101.6 ± 10.1	100.2 ± 11.1	94.0 ± 8.0	103.6 ± 12.3	0.043	
MMEF (% predicted)	105.7 ± 28.6	106.7 ± 24.8	100.9 ± 22.8	109.8 ± 32.3	0.880	
Bronchodilator response (%)	6.0 ± 8.8	5.3 ± 6.1	3.1 ± 8.4	7.2 ± 7.8	0.516	

All P values for the multiple comparisons were < 0.008 (the adjusted P value based on the Bonferroni correction). Data are presented as mean ± standard deviation.
FEV$_1$, forced expiratory volume in 1 second; FVC, forced volume vital capacity; MMEF, maximal mid-expiratory flow.

Changes in lung function variables from baseline to 6 months across clusters

The results of pulmonary function tests during a follow-up period of 6 months are shown in **Table 5**. Similar to the results of initial pulmonary function tests, all pulmonary function variables were preserved 6 months later. FEV$_1$ in cluster 3 increased from 84.6% to 91.4%, likely reflecting an improvement in the degree of airway obstruction after enrollment in this study. However, prebronchodilator FVC (93.8 ± 8.6%), postbronchodilator FEV$_1$ (92.8 ± 8.3%), and FVC (94.0 ± 8.0%) remained significantly lower in cluster 3 than in the other clusters.

DISCUSSION

To the best of our knowledge, this is the first nationwide study showing that asthma phenotypes in Korean children could be classified into 4 distinct clusters: cluster 1 comprising male-dominant atopic asthma: cluster 2, early-onset atopic asthma with AD: cluster 3, puberty-onset, female-dominant atopic asthma: and cluster 4, early-onset, non-atopic dominant asthma. These findings demonstrated that childhood asthma could be classified into distinctive phenotypes, consistent with the clinical characteristics partly revealed in previous studies.[6,7,26,27]

Several previous studies on asthma clusters have demonstrated the heterogeneity of childhood asthma.[7,9,11,13,15,20] In the Severe Asthma Research Program (SARP) cohort, asthma duration, number of asthma controller medications, and baseline lung function were major determinants of the asthma phenotype in cluster analysis. Because this study recruited only patients with severe asthma, all of those with childhood asthma had atopic disease, with reduced lung function.[13] As our present study recruited and analyzed a nationwide cohort of Korean patients with childhood asthma, the percentage of patients with severe asthma was lower than the percentages of patients with mild persistent and mild intermittent asthma. The rates of hospitalization and ED visits were low and showed no differences across the clusters. The rates of severe asthma did not differ significantly among the 4 clusters. Almost all children in the KAS cohort showed preserved lung function. These differences were probably due to the differences in asthma severity and ethnicity between the SARP cohort and ours.[28] Additionally, the SARP cohort did not include any patients with a low degree of atopy, whereas a few patients in cluster 4 in the present study had atopic features.

In the present study, cluster 1 comprised patients with clinically typical early-onset childhood asthma with allergic sensitization, a condition that affects boys more than girls in childhood. Male dominance and atopy were key characteristics as previously reported.[1,9,29,30] The clinical course of these patients with a typical childhood asthma phenotype in Korea will be followed

up in the future. Cluster 2 was characterized by a significantly high rate of lifetime history of AD diagnosis (98.7%) and current AR diagnosis (86.1%), with early-onset asthma, indicative of atopic march. Compared with the other groups, however, AD was not a risk factor for more exacerbations or less well-controlled asthma. Rather, children in cluster 2 showed relatively mild symptoms of asthma, with fewer using controller medications. Previous study results have differed regarding the association between AD and asthma severity. For example, oral corticosteroid use and ED visits were significantly higher in children with than without eczema symptoms.[31] However, other studies suggested that early AD alone did not increase the risk of current wheeze and BHR in 7-year-old children, and that only 3.1% of children with asthma showed allergic march.[32,33] Furthermore, the genetic susceptibility regions for asthma and AD showed little overlap, suggesting that different genes may be involved in the pathogenesis of these atopic disorders.[34] AD may not be associated with asthma severity, suggesting the need for further evaluation and assessment.

Cluster 3 in this study, characterized by puberty-onset, female-dominant atopic asthma, showed the most frequent asthma symptoms, including relatively lower lung function, among all clusters. Because patients in this cluster were older than those in the other clusters, with a high proportion having entered puberty, patients in the other clusters have the potential to enter cluster 3 with age. However, this cluster seems to be a distinct group rather than a progression from other clusters, because the patients were older at asthma onset than those in the other groups. Sex hormones may be associated with the development of asthma symptoms during puberty, suggesting that sex hormones may play an important role in the pathogenesis of asthma.[35] Dysanaptic airway growth may also partially account for the physiological differences between men and women, because the small airway resistance is greater in women than in men.[36] Recent studies explain that the androgen surge with puberty is likely to confer protective effects on lung growth in both males and females.[37,38] However, estrogens may have deleterious effects on lung growth in females extending into adult development.[37,39] Although the exact mechanisms are unclear, asthma symptoms were more predominant and severe in female than male adolescents at the onset of puberty, indicating that puberty is a critical stage in asthma symptom progression.[26] These studies may help explain the female dominant asthma phenotype during adolescence, and likewise inform lung growth and asthma severity with subsequent maturation into adulthood. Thus, attention should be paid to newly diagnosed asthma in adolescent girls, with asthma control and lung function assessed regularly. Furthermore, this cluster showed the highest BMI among the 4 groups, although there was no statistical significance in the analysis with BMI percentile considering their age. These findings may help predict more important variables in patients with persistently uncontrolled asthma. Interestingly, children in cluster 3 showed the low-lung function, as assessed by FVC and FEV_1, both at baseline and after bronchodilator administration. This group also showed the same tendency of the low lung function after 6 months as those at baseline. These pulmonary function results are specific characteristics of cluster 3, which may be associated with an air-trapping phenotype.[40,41] This can only be interpreted as a characteristic of cluster 3, but we can carefully interpret this result to indicate that the baseline cluster's characteristic is maintained during a period of 6 months. Therefore, it suggests that cluster analysis is divided into groups with their distinct characteristics rather than temporal cross-sectional statistical results. Further studies are required to assess the role of sex hormones and sex-specific dysanaptic airway growth, and the effects of obesity on pulmonary physiology, immunology, and pathology of asthma.

Airway hyper-responsiveness is considered a marker of asthma, independent of the atopic status, and it should be considered a parallel pathological process that can lead to subsequent

symptoms and clinical evidence of asthma in children without evidence of atopy. Non-atopic asthma has a milder and shorter prognosis than atopic asthma.[42] In the present study, the early-onset non-atopic dominant asthma group (cluster 4) showed the second highest asthma severity among the 4 clusters. Furthermore, several of these patients required combination therapy, probably because this cluster included some patients with atopy and those with non-atopic asthma suffering from neutrophil-dominant steroid-refractory recurrent wheeze,[43,44] which was previously reported in other studies. These findings suggest that the prognosis of patients with non-atopic dominant early-onset asthma varies among the clusters. Longitudinal monitoring of this group may reveal factors common to adults with non-atopic asthma.

This study had several notable limitations. Because the current cluster analysis was based on cross-sectional data, with pulmonary function monitored after 6 months, it is necessary to determine whether these 4 clusters show dynamic changes across clusters or are fixed at follow-up. Long-term follow-up, which includes assessment of treatment patterns and responses to treatment, is also required. Another limitation may be the small sample size and the relatively short-term follow-up period in this study. Assessments of larger patient populations are required to identify critical variables, including disease subtypes, environmental factors, and biomarkers, which would allow the differentiation of asthma subtypes at any cross-sectional time point. Additionally, we evaluated asthma onset and history of acute bronchiolitis based on parent-reported questionnaires dependent on remote memory recall, suggesting a possibility of reporting bias. Nevertheless, this method is commonly used in epidemiological research on children, and the questionnaires we used have proven reliable and been validated in previous studies.[45] This is the first nationwide prospective childhood asthma cohort study in Korea. Because we evaluated childhood asthma using well-validated measures, including pulmonary function tests, methacholine tests, skin prick tests, and serum total IgE concentrations in patients with physician-diagnosed asthma, the results represented a real-world assessment of childhood asthma in Korea. Finally, the participants in the current study will be evaluated every 6 months, enabling long-term predictions of prognosis and response to treatment in children with various asthma phenotypes.

In conclusion, the asthma phenotype in Korean children can be classified into 4 distinct clusters. Further challenges to the optimal use of clustering methodologies include tailoring models to individual data sets as well as incorporating genetic, epigenetic, and more detailed molecular-level data.

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIALS

Supplementary Table S1
Results of stepwise linear discriminant analysis of the 12 variables used in cluster analysis

Click here to view

Supplementary Table S2

Baseline characteristics of total subjects (n = 674) and subjects who are included and not included in cluster analysis

Click here to view

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