

Atopic dermatitis phenotype with early onset and high serum IL-13 is linked to the new development of bronchial hyperresponsiveness in school children

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Abstract

Background: Atopic dermatitis (AD) is characterized by a heterogeneous clinical spectrum, and some forms of AD are associated with the initial steps of allergic march. The aims of this study were to determine AD phenotypes in school-age children and investigate their associations with the allergic march in each cluster.

Methods: We included 242 children (6–8 years) with current AD from the Children's Health and Environmental Research study, a 4-year prospective follow-up study with 2-year survey intervals. Latent class analysis was used. Serum IL-13 and thymic stromal lymphopoietin (TSLP) levels at the time of enrollment were measured using ELISA.

Results: We identified four current AD phenotypes in children, characterized as 'early onset with low atopy' (26.4% of the sample; group 1), 'early onset with high atopy and high eosinophil percentages' (48.3%; group 2), 'late onset with low atopy' (9.9%; group 3), and 'late onset with high atopy and normal eosinophils' (15.3%; group 4). Although groups 2 and 4 demonstrated high atopic burden, children in group 2 showed the persistence of AD and eosinophilia associated with a high prevalence of new cases of bronchial hyper-responsiveness and asthma symptoms during follow-up. The serum IL-13 level was significantly increased in the early-onset AD groups, but there was no significant difference in the serum TSLP levels across all four groups.

Conclusion: An allergic march-associated AD phenotype exists that is characterized by early onset of AD with its persistence, increased serum IL-13 levels, high atopy, and a persistently increased blood eosinophil percentage.

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritus and a relapsing clinical course (1). The prevalence of AD has increased in recent decades to 15–30% in children, but there are regional differences (2, 3). Atopic dermatitis affects the quality of life and imposes a large socioeconomic and familial psychosocial burden (2, 4, 5). In previous prospective studies, AD was associated with

an increased risk of childhood asthma (6–9), which represents the allergic march. Approximately one-third of children with AD develop asthma later in life (10), and many asthmatic children present wheezing before or simultaneously with the onset of AD (11).

Studies on the natural course of AD have evaluated the factors associated with the later development of asthma in

children with AD (11), but these studies lacked repetitive objective assessment of asthma in the characterization of allergic march-associated AD. Studies on the classification of AD according to the age at onset of AD or immunoglobulin E (IgE) association have also been performed (12, 13), but these studies were limited by observer bias in the artificial classification of AD.

The underlying mechanisms of the allergic march remain unclear, but animal studies suggest that IL-13 and thymic stromal lymphopoietin (TSLP) are the core pathophysiologies of allergic march (14–16). Signals from skin inflammation in AD may act as triggers of the allergic march and thereby promote sensitization to inhalant allergens, which are risk factors for asthma and allergic rhinitis (AR) (17). Although systemic Th2 immune responses are considered to be the fundamental mechanisms of the allergic march, there have been few studies on these issues in affected patients.

Cluster analysis of AD is a useful approach for classifying the diverse clinical spectrum of the disorder according to similar characteristics and for understanding the connection between specific AD phenotypes and their associations with other allergic diseases. Compared with artificial classifications of AD, an unsupervised cluster analysis without observer bias might better reflect the complex heterogeneity of AD and thereby provide important clues for identifying the underlying pathophysiology of the allergic march.

The principal aim of this study was to define AD phenotypes in children aged 6–8 years using a latent class analysis (LCA) that focused on the causative factors of AD development. In addition, we investigated whether the AD phenotype was linked to the new development of bronchial hyper-responsiveness (BHR) and asthma. Serum IL-13 and TSLP levels were measured to identify the possible underlying pathophysiologies of the allergic march in humans.

Methods

Study population

This study forms part of the Children's HEalth and Environmental Research (CHEER) study, which aims to evaluate the association between environmental factors and children's health, including allergic diseases (18–20). The study population was enrolled between 2005 and 2006 at the age of 6–8 years. Because the characteristics of AD affect the natural course of the allergic march and objective assessment of asthma is possible in children older than 6 years, we included children in this age group with current AD. Two prospective follow-up surveys were performed at 2-year intervals. Of a total of 2491 children who underwent skin prick tests (SPTs), 461 had current AD. After excluding 219 of these children for whom data on the age at onset of AD were missing, a total of 242 children were included in the study (Fig. S1). 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 (IRB) of Ulsan University College of Medicine (IRB no. 2006-0081).

Questionnaire

The Korean version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to investigate asthma, rhinitis, and eczema (19, 21). Parents or guardians completed the questionnaire, which includes the following items: (i) general characteristics such as sex, age, height, and weight; (ii) history of AD diagnosis by physicians or AD treatment in the last 12 months, age at parent-reported doctor-diagnosed AD, and history of doctor-diagnosed asthma; and (iii) household income and environmental factors, such as exposure to environmental tobacco smoke (ETS).

SPTs and total serum immunoglobulin E (IgE)

Skin prick tests were performed for the 13 most common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog dander, cat epithelium, cockroaches, *Alternaria alternata*, *Aspergillus fumigatus*, grass pollen mixture, tree mixture, ragweed, mugwort, alder, and oak) and four food allergens (peanuts, egg whites, cow's milk, and soybeans) (19). Histamine (10 mg/ml) was used as the positive control, and normal saline was used as the negative control. A mean wheal size ≥ 3 mm when measured after 15 min and wheals caused by histamine were considered positive. Total serum IgE levels were measured using the Immunocap-CAP 1000 system (Aloka, Tokyo, Japan). The lower detection limit for total serum IgE was 2 kU/l.

Lung function tests and BHR

Baseline lung function, including forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), and forced expiratory flow at 25–75% of FVC (FEF_{25–75%}), was assessed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (19, 20, 22). Bronchial hyper-responsiveness was assessed during 1 day by all participants via a methacholine bronchial challenge using tidal inhalation of doubling methacholine doses (0.625–25 mg/ml: 0.625, 1.25, 2.5, 5, 10, and 25 mg/ml), and the cumulative methacholine dose that caused a 20% reduction in FEV₁ (PC₂₀) according to ATS recommendations was recorded (23). Participants were considered to have BHR to methacholine when the methacholine PC₂₀ was ≤ 8 mg/ml.

Definitions of atopy, current AD, asthma, and new development of BHR

Atopy was defined by a positive skin reaction to ≥ 1 allergen on the SPTs. Current AD was defined as parent-reported, doctor-diagnosed AD and eczema in the last 12 months. Physician-diagnosed asthma was assessed by the question 'Has your child ever been diagnosed with asthma by physicians?' Newly diagnosed asthma was defined as a conversion from no history of physician-diagnosed asthma at the time of enrollment to the presence of physician-diagnosed asthma during follow-up. Newly developed BHR was defined as

conversion from a negative BHR (>8 mg/ml) at enrollment to a positive BHR (≤ 8 mg/ml) during the prospective 4-year follow-up period.

Cluster analysis and variables selection

An LCA was used to identify the current AD phenotypes. For the LCA, we included 12 variables associated with the causative factors of AD, such as personal characteristics (sex and body mass index), socioeconomic status (income and maternal education levels), environmental factors (e.g., exposure to ETS), parental history of any allergic diseases, comorbidities of other allergic diseases at enrollment, age at onset of AD, AD treatment in the previous 12 months, and SPT and blood test results (including total serum IgE levels and eosinophil percentages). To better reflect the causative factors from the questionnaire, diverse variables related to socioeconomic and environmental factors were included in the analysis. Continuous variables were dichotomized. To determine the optimal number of clusters, we selected the lowest Bayesian information criterion (24), which was the model of the four groups included in this study.

To validate the phenotypes and evaluate the prognosis in each cluster, we assessed lung function and BHR at enrollment; each cluster subsequently received follow-up examinations for 4 years at 2-year intervals using SPTs, lung function tests, BHR assessment, biomarkers, and questionnaires covering the same items assessed at enrollment.

Serum IL-13 and TSLP levels

Blood samples were obtained from the study participants in the first survey. The control group was selected from participants in the total study population who satisfied the following criteria: participants without any allergic diseases or

family history of allergic diseases including AD, AR, and asthma, a negative BHR, $<4\%$ of serum eosinophils, and <100 kUA/l of total serum IgE levels in all surveys. The serum levels of IL-13 (control group, $n = 19$; group 1, $n = 9$; group 2, $n = 21$; group 3, $n = 6$; and group 4, $n = 10$) and TSLP (control group, $n = 21$; group 1, $n = 9$; group 2, $n = 20$; group 3, $n = 6$; and group 4, $n = 10$) were measured in randomly selected samples from patients who provided consent using a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All samples were analyzed in duplicate.

Statistical analysis

To compare the differences in characteristics among the four groups, ANOVA, Kruskal–Wallis, or chi-square tests were used for parametric continuous, nonparametric continuous, or categorical variables as appropriate. A Bonferroni correction was performed after the Kruskal–Wallis test. The associations between specific environmental factors and each phenotype were analyzed using a logistic regression analysis and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Adjustments were made for potential confounding factors, such as sex, age, area, income, and family history of allergic diseases. $P < 0.05$ was considered significant. All statistical analyses were performed using SAS for Windows (version 9.2; SAS Institute Inc., Cary, NC, USA).

Results

Participant characteristics

The baseline characteristics of the 242 children with current AD are presented in Table 1. The mean age of the study participants was 7.54 years, and 51.7% were male. Among

Table 1 Baseline characteristics of the children included and excluded from this study

Variables	Included in this study ($n = 242$)	Children with missing information regarding age at the onset of AD ($n = 219$)	Excluded from this study ($n = 2249$)
Age, mean $\pm$ SD (years)	7.54 $\pm$ 0.93	7.44 $\pm$ 0.82	7.69 $\pm$ 1.14
Sex			
Male, n (%)	125 (51.7)	108/219 (49.3)	1172 (52.1)
Body mass index (kg/m^2), mean $\pm$ SD	16.72 $\pm$ 2.18	16.96 $\pm$ 2.61	16.90 $\pm$ 2.59
Parental history of any allergic diseases (AD, AR, or asthma)	106/241 (43.8)	82/204 (40.2)	573/2060 (27.8)†
Household income (monthly)			
<3000 USD, n (%)	153 (64.3)	146/216 (67.6)	2124 (94.4)†
Atopy at first survey, n /total (%)	79/219 (36.1)	56/117 (47.9)*	473/1473 (32.1)
Methacholine PC ₂₀ < 8 mg/ml at first survey, n /total (%)	42/231 (18.2)	64/214 (29.9)*	446/2194 (20.3)
Total serum IgE (kU/l) at first survey, mean (range)	326.51 (3–5521)	341.36 (3–3840)	263.64 (2–6934)
Eosinophil percentage at first survey, mean (range)	4.12 (0–18)	4.05 (0–17)	3.62 (0–39)†

AD, atopic dermatitis; AR, allergic rhinitis; n , number; USD, US dollars; SD, standard deviation.

* P value < 0.05 between the 242 children included in this study and 219 children excluded due to missing information regarding age at the onset of AD.

† P value < 0.05 between the 242 children included in this study and 2249 children excluded due to no current AD at the time of enrollment or missing information regarding age at the onset of AD.

the participants, 43.8% had a parental history of allergic diseases, including AD, AR, or asthma. At enrollment, 36.1% exhibited atopy and 18.2% had a positive BHR.

Latent cluster characteristics

Four clusters of current AD were identified: group 1, early onset with low atopy; group 2, early onset with high atopy and high eosinophil percentage; group 3, late onset with low atopy; and group 4, late onset with high atopy and normal eosinophil percentage (Table 2). Group 1 (26.4% of the cohort) was characterized by a relatively early onset of AD, a high prevalence of nonatopy, low levels of total serum IgE, and a normal eosinophil percentage. The largest group (group 2; 48.3% of the cohort) was characterized by early-onset AD with a high prevalence of atopy, high levels of total serum IgE, and high serum eosinophil percentage. A tree analysis to assess the classification of subjects by subsets of variables used in the LCA is presented in Fig. S2. Most participants in group 2 had a normal body mass index and a high prevalence of parental history of allergic diseases and comorbidities of allergic diseases, including AR and asthma at enrollment. Group 3, the smallest group (9.9% of the

cohort), was characterized by late-onset AD with low levels of total serum IgE and a normal eosinophil percentage. The prevalence of parental history of allergic diseases and comorbidities of allergic diseases were low. In addition, this group included participants with a low household income and low maternal education levels. Participants in group 4 (15.3%) were characterized by late-onset AD with a high prevalence of elevated total serum IgE levels and a normal eosinophil percentage. Except for one participant in group 4, none had any parental history of allergic diseases and most had a low maternal education level. In the highly atopic groups, namely groups 2 and 4, participants in group 4 were more likely to be overweight than participants in group 2 (group 2, 3%; group 4, 49%). Groups 3 and 4 included children of a low socioeconomic status. There were no significant differences in

Table 2 Characteristics of the AD phenotypes

Group	1 N (%)	2 N (%)	3 N (%)	4 N (%)
Subject number	64 (26.4)	117 (48.3)	24 (9.9)	37 (15.3)
Male	12 (19)	87 (74)	17 (71)	9 (24)
Body mass index				
Normal	52 (81)	113 (97)	23 (96)	19 (51)
Parental history of allergic diseases				
Yes	34 (53)	65 (56)	3 (13)	1 (3)
Comorbidities of allergic diseases				
Yes	27 (42)	68 (58)	3 (13)	9 (24)
Monthly income				
<3000 USD	34 (53)	68 (58)	24 (100)	17 (46)
Maternal education levels				
Less than high school	27 (42)	56 (48)	19 (79)	35 (95)
Exposure to ETS				
Yes	42 (66)	51 (44)	8 (33)	15 (41)
Age at AD diagnosis				
<1 year	29 (45)	48 (41)	1 (4)	3 (8)
1–3 years	15 (23)	30 (26)	9 (38)	13 (35)
3–6 years	17 (27)	29 (25)	13 (54)	21 (57)
≥6 years	3 (5)	10 (8)	1 (4)	0 (0)
AD treatment in the last 12 months				
Yes	44 (69)	86 (74)	24 (100)	37 (100)
Positive skin reaction on skin prick tests				
≥1 allergen	2 (3)	65 (56)	4 (17)	4 (11)
Total serum IgE levels				
≥100 kU/l	8 (13)	100 (85)	0 (0)	31 (84)
Serum eosinophil				
≥4%	8 (13)	84 (72)	0 (0)	12 (32)

AD, atopic dermatitis; ETS, environmental tobacco smoke; USD, US dollars.

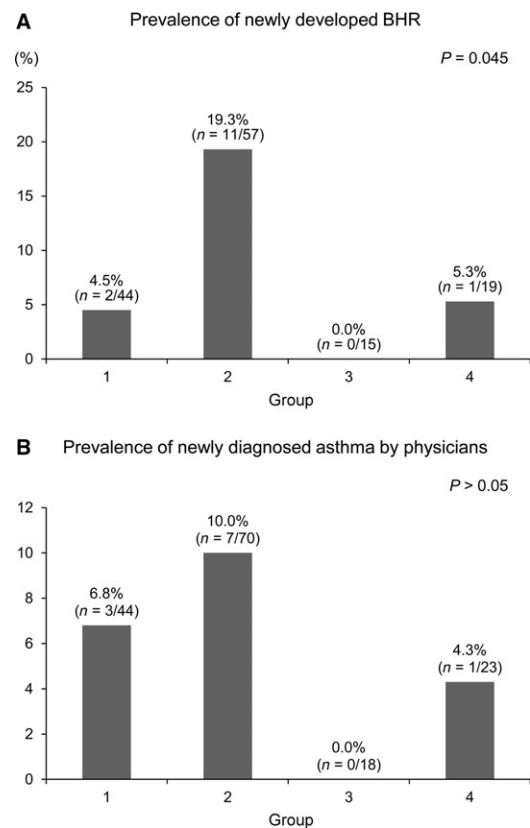


Figure 1 Changes in bronchial hyper-responsiveness (BHR) during the prospective follow-up period according to atopic dermatitis (AD) phenotype. (A) Prevalence of newly developed BHR during the 4-year follow-up period in each group (group 1, $n = 2/44$; group 2, $n = 11/57$; group 3, $n = 0/15$; group 4, $n = 1/19$). (B) Prevalence of newly diagnosed asthma by physicians during the 4-year follow-up period in each group (group 1, $n = 3/44$; group 2, $n = 7/70$; group 3, $n = 0/18$; group 4, $n = 1/23$). PC₂₀ was defined as the provocative methacholine concentration resulting in a 20% decrease in FEV₁. Mean PC₂₀ was calculated among children with methacholine provocative concentration <8 mg/ml that caused a 20% decrease in FEV₁ (PC₂₀). P was assessed using the Kruskal–Wallis test.

the prevalence of parental-reported, doctor-diagnosed food allergy among the four groups (data not shown).

Prognosis

In comparison with group 1, only group 2 demonstrated inverse associations with AD remission during follow-up (adjusted OR = 0.310; 95% CI = 0.115–0.832). The prevalence of BHR at enrollment was significantly higher in group 2 (25.5%) than in the other groups ($P = 0.017$). During follow-up, newly developed BHR was significantly more prominent in group 2 (19.3%) than in the other groups ($P = 0.045$; Fig. 1A). The prevalence of new physician-diagnosed asthma during follow-up was highest in group 2 (10.0%), but this difference was not statistically significant (Fig. 1B). Regarding total serum IgE levels and eosinophil percentages, significant differences were observed between groups 1 and 2 (Fig. 2). Group 2 demonstrated the most prominent atopy at the time of enrollment and follow-up, even among patients with multiple sensitizations (Fig. 3). The prevalence of new

sensitization on SPTs increased after 3 years of follow-up in all groups, with the highest increase noted in group 2 (data not shown).

Serum IL-13 and TSLP levels

In comparison with the control group, serum IL-13 levels were significantly elevated in groups 1 and 2, which included children with early-onset AD (control group vs group 1, $P = 0.035$; control group vs group 2, $P = 0.025$; Fig. 4A). There were no significant differences in serum TSLP levels compared with the control group or across the AD groups (Fig. 4B).

Discussion

Using unsupervised cluster analysis, we identified four distinct AD phenotypes in school-age children from a general population-based cohort. Children with current AD were divided into two subgroups according to their age at onset of

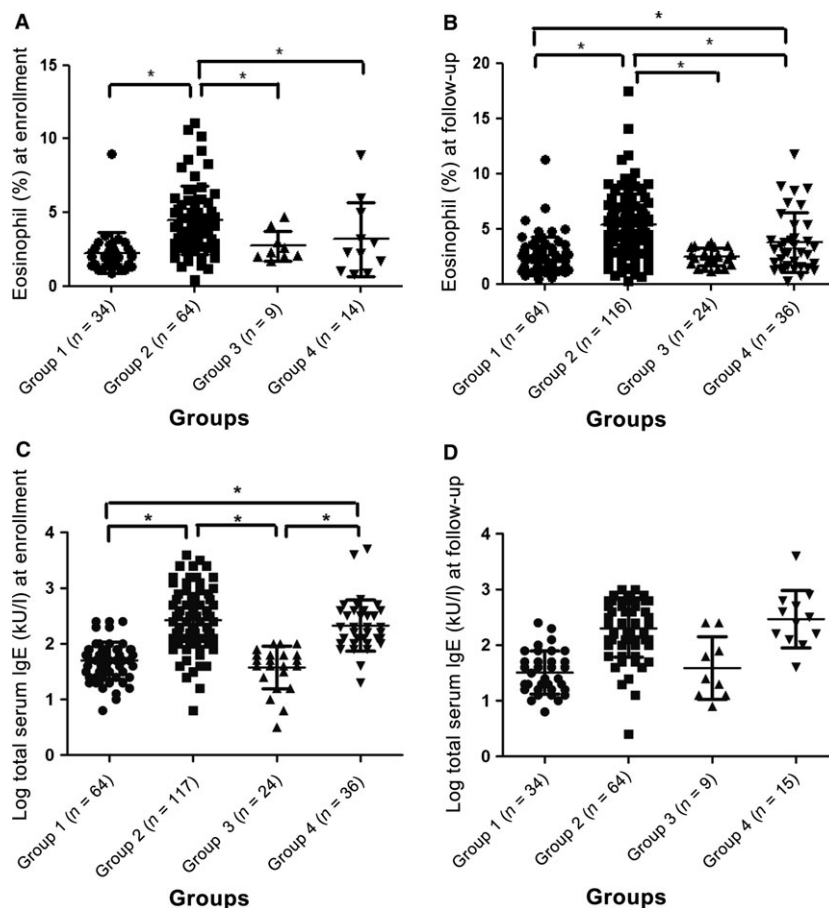


Figure 2 Differences in total serum IgE levels and eosinophil percentage in each group at enrollment and the 4-year follow-up examination. (A) Mean and standard deviation of serum eosinophil percentage at enrollment. (B) Mean and standard deviation of the serum eosinophil percentage at follow-up. (C) Mean and standard

deviation of log total serum IgE levels at enrollment. (D) Mean and standard deviation of log total serum IgE levels at follow-up. Data are shown as the mean and standard deviation. P was calculated using Kruskal–Wallis test or Mann–Whitney U -test, as appropriate. * $P < 0.05$.

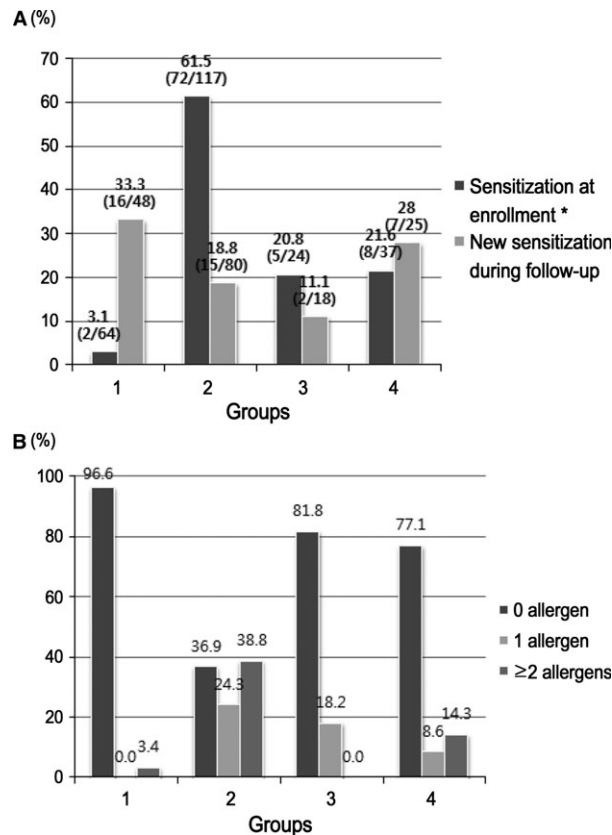


Figure 3 Prevalence of atopy in each study group. (A) Prevalence of atopy at enrollment (group 1, $n = 2/54$; group 2, $n = 72/117$; group 3, $n = 5/24$; group 4, $n = 8/37$) and the new development of atopy at the 4-year follow-up examination (group 1, $n = 16/48$; group 2, $n = 15/80$; group 3, $n = 2/18$; group 4, $n = 7/25$). (B) Prevalence of multiple sensitizations against at least two allergens at enrollment across all four groups (group 1, $n = 2/59$; group 2, $n = 40/130$; group 3, $n = 0/22$; group 4, $n = 5/35$). * $P < 0.001$ was calculated from Kruskal–Wallis tests for the differences in the prevalence of atopy across the study groups.

AD. The two subgroups were further subdivided based on atopic burden, as reflected by total serum IgE levels and atopy. Children with early-onset AD and high atopic burden (group 2) demonstrated a persistently high eosinophil percentage during follow-up, whereas children with late-onset AD and high atopic burden (group 4) exhibited a normal eosinophil percentage. In addition, compared with the control group, children with early-onset AD (groups 1 and 2) demonstrated significantly higher serum IL-13 levels at the time of enrollment. Children in group 2 showed a high prevalence of newly developed BHR and physician-diagnosed asthma and earlier and multiple sensitizations. This group also had a significantly lower AD remission rate. The results of this study suggest the presence of the allergic march-associated AD phenotype, which demonstrates the features of early-onset and persistent AD, high atopic burden, a persistently increased percentage of eosinophils, and increased

serum IL-13 levels. However, the lack of significant differences in the prevalence of food allergy across the four phenotypes might suggest the presence of independent food allergy-associated AD phenotypes with high risk of the allergic march. Further studies on these phenotypes are required. Our present study is the first demonstration of allergic march-associated AD phenotypes in school-age children and the first identification of the characteristics of these individuals and possible underlying pathophysiologies in a prospective study.

Although there are a number of studies on asthma phenotypes (25–27), only a few reports have investigated AD classification (12, 13). Because LCA can identify separate subclasses with similar characteristics on the basis of the observed heterogeneity (28) and has the virtue of greater objectivity than the previously described phenotypes, previous studies on asthma phenotypes have used this method (25, 29). Therefore, this method has the virtue of greater objectivity compared to those previously described phenotypes. Because LCA can identify the distinct subclasses of disease entities, the method was previously applied to cluster asthma phenotypes (25, 29). However, no previous study has used LCA to classify AD phenotypes. One of the studies on AD phenotypes artificially classified 184 AD children with a median age of 36 months into two groups based on the association with IgE in a hospital-based population, and the patients and their natural courses were retrospectively evaluated using medical records for 10 years. In addition, children with IgE-associated AD were divided into two subgroups according to the presence of respiratory allergic symptoms (12). However, that study lacked an assessment of the sequential development of asthma or AR in relation to AD using objective indicators and definitions for the diagnosis of asthma. Although IgE-mediated immune responses might predispose a patient to allergic march, the classification of AD only according to IgE levels does not explain the underlying mechanisms of allergic march (30, 31). Another study evaluated the risk factors for the development of AD depending on onset age (13), but that study did not establish a link between different onset age of AD and the underlying pathophysiologies. On the other hand, in our present study, we aimed to combine not only biomarkers, but also various possible causative factors such as environmental and genetic factors, to unravel the underlying pathophysiology of specific AD phenotypes. Our present analysis also evaluated the prognosis of each phenotype using objective allergic tests during the prospective follow-up. By analyzing the diverse spectrum of AD phenotypes, we found that not only total serum IgE levels, but also eosinophil percentages and serum IL-13 levels may be associated with allergic march-associated AD phenotypes in school-age children.

Based on our current findings, we speculate that eosinophils may contribute to the progression of allergic march. The chemokines or cytokines involved in the differentiation, recruitment, and proliferation of eosinophils may be implicated in the pathogenesis of allergic march, either as potent inducers of Th2 immune responses or as the chronic inflammation underlying this condition (14, 32–34). Eosinophils

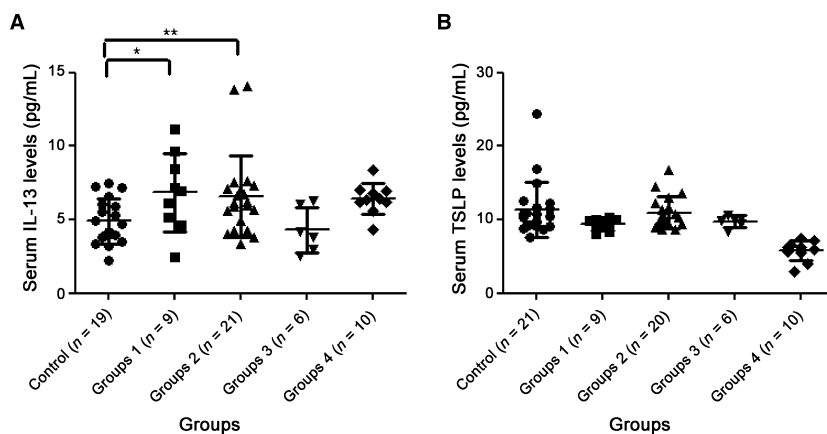


Figure 4 Serum IL-13 and thymic stromal lymphopoietin (TSLP) levels at the time of enrollment for each group. (A) Early-onset atopic dermatitis (AD) groups, including groups 1 and 2, demonstrated significantly higher serum IL-13 levels in comparison with the con-

trol group. (B) There were no significant differences in serum TSLP levels across all four study groups in comparison with the control group ($P > 0.05$). $*P = 0.035$, $**P = 0.025$. P was calculated using Kruskal–Wallis test or Mann–Whitney U -test, as appropriate.

play a key role in the proinflammatory process, tissue repair, and fibrotic processes in allergic diseases (35). Some of the intracellular components of eosinophils are involved in the cascade of allergic inflammatory reactions. For example, neurotoxin, which has the function of alarmin in the cytoplasm of eosinophils, activates dendritic cells and thereby leads to Th2 immune responses.

Previous studies have shown that eosinophils are involved in the development of BHR (36) and allergen-induced pulmonary pathology (37), although the role of eosinophils in allergic march has not been fully characterized. Eosinophils can produce and release functional IL-13 in inflammatory responses (38). IL-13 inversely acts as a potent inducer of eosinophil-activated chemokines, including eotaxin and RANTES (39). The transgenic expression of IL-13 in the skin can cause remarkable infiltrations of inflammatory cells with increased Th2 cytokines, even in the absence of epicutaneous allergen sensitization (40). Th2 cytokines, such as IL-4/IL-13, induce the production of periostin, which is linked to the Th2 response, keratinocyte activation, and subepithelial fibrosis in AD and bronchial asthma (41, 42). Eosinophil levels reflect the severity, activity, and development of AD (35, 43, 44), which in this study were found to be associated with the lowest AD remission rate in group 2. Taken together, our current data suggest that eosinophils and related cytokines involved in the differentiation, recruitment, and proliferation of eosinophils may play a potent role in the underlying pathophysiology of allergic march, even in humans (45).

Thymic stromal lymphopoietin has been considered a key mediator in the pathogenesis of allergic march in animal studies (14), but we could not find significant differences in TSLP levels across AD phenotypes in our present study. Although this might be partially attributable to the small sample size, limited availability of samples, and more accurate reflection of the development of allergic march in tissue TSLP levels than in serum TSLP levels, further studies on this issue are required (14).

The allergic march-associated AD phenotype demonstrated a high prevalence of atopy at the time of enrollment in our present analyses. In addition to previous studies, which demonstrated allergen sensitization as a risk factor for allergic march (46, 47), our current study indicates that sensitization in early life may be associated with the development of allergic march in AD children. Although sensitization to environmental allergens is not a simple cause-and-effect relationship in allergic march (48, 49), this process could be an integral part of the immunopathogenic mechanisms involved in the progression of allergic march, in addition to increases in IL-13 and eosinophils (50).

One of the strengths of our present report was that it is a general population-based longitudinal study, thereby reflecting a real-world situation wherein general physicians encounter AD children. A prospective German birth cohort study evaluated the natural course of infant AD and its association with asthma at the age of 7 (11). That study enrolled participants with high risk of allergic diseases and analyzed the associated factors with asthma development using artificial classifications. On the other hand, our study applied unsupervised methods to classify the AD phenotypes and prospectively performed follow-up examinations. We also clustered the clinical heterogeneity of current AD in combination with socioeconomic and demographic characteristics. To validate the underlying pathophysiology of an allergic march, we measured serum IL-13 and TSLP levels. In addition, we tried to objectively assess asthma using pulmonary function tests and BHR.

Our study had some limitations of note. First, the sample size was relatively small because we included children with current AD from a general population. Considering that 85% of childhood AD develops before the age of 5 and mainly improves with age (11), the prevalence of school-age children with current AD is low. However, our current analyses suggested the presence of allergic march cases in schoolchildren with AD. Second, some of our participants were lost during follow-up. However, the follow-up rate of

the patients in our present series was relatively high at approximately 75% (20). Third, we did not examine the symptom severity of AD because we used the ISAAC questionnaire. There is considerable temporal diversity in the severity of AD, even within an individual, and evaluating the severity at one time-point does not reflect the whole severity of AD. Because total serum IgE levels, tissue eosinophilia, and allergic sensitization are partially correlated with the severity of AD (35, 51), the AD severity might be reflected in the classifications. Although biomarkers were measured in only a portion of each subgroup due to the limited availability of blood samples, there were no significant differences in the characteristics between participants with and without blood samples for the analysis of serum IL-13 and TSLP. Because the serum IL-13 and TSLP levels were measured in a relatively small portion of the study population, further studies in a larger sample are required to confirm the results of this study. However, the values of this study lie in its identification of the characteristics of allergic march-associated AD phenotypes, especially in school-age children.

Because the CHEER study enrolled children at the age of 6–8 from a general population and followed up with them until they were 10–12 years, our present analysis was also limited in terms of the identification of the natural course of AD during early life. However, there have been no previous studies on the natural course of AD in school-age children and its association with the allergic march. Our current findings can therefore provide important clues for identifying the underlying pathophysiology of the allergic march. Because our study population was randomly selected from elementary schools nationwide, our results are representative of AD phenotypes in schoolchildren.

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Conflicts of interest

There is no conflict of interest to declare.

Supporting Information

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

Figure S1. Flow chart describing the enrollment of the study population.

Figure S2. Tree analysis by variables used in the latent class analysis identified four subgroups of atopic dermatitis.

16. Kim HJ, Kim YJ, Lee SH, Yu J, Jeong SK, Hong SJ. Effects of *Lactobacillus rhamnosus* on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4(+)CD25(+)Foxp3(+) Tregs. *Clin Immunol* 2014;**153**:178–186.
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Enrollment at first survey (n = 2491)

Children with current AD (n = 461)

Exclusion due to missing information regarding
age at onset of AD (n = 219)

Study population (n = 242)

