

ORIGINAL ARTICLE

Phenotypes of atopic dermatitis identified by cluster analysis in early childhood

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ABSTRACT

Atopic dermatitis is a chronic, relapsing, inflammatory skin disease that usually appears in early childhood and develops into a heterogeneous disease during childhood. The clinical course and treatment for atopic dermatitis can differ according to its phenotype and/or endotype. This study aimed to identify clinical phenotypes of atopic dermatitis in early childhood. Data were obtained from 572 children under 3 years of age with atopic dermatitis. Cluster analysis applied to 11 variables, and we identified four clusters of atopic dermatitis. Children in cluster A ($n = 141$) had early-onset atopic dermatitis with high blood eosinophil counts, serum total immunoglobulin E and rates of sensitization to food allergens. Children in cluster B ($n = 218$) had early-onset atopic dermatitis with low blood eosinophil counts, serum total immunoglobulin E and rates of sensitization to both food and inhalant allergens. Children in cluster C ($n = 53$) had early-onset atopic dermatitis with high C-reactive protein levels and white blood cell counts. Children in cluster D ($n = 160$) had middle-onset atopic dermatitis with high serum total immunoglobulin E and rates of sensitization to inhalant allergens. Cluster A had the highest Scoring for Atopic Dermatitis and transepidermal water loss values. Age at onset, age at diagnosis, white blood cell count, eosinophil count, C-reactive protein and serum total immunoglobulin E level were the strongest predictors of cluster assignment. Analysis of these six variables alone resulted in correct classification of 95.5% of the subjects. These results support the heterogeneity of atopic dermatitis, even in early childhood.

Key words: atopic dermatitis, child, preschool, cluster analysis, infant, phenotype.

INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease.¹ It usually develops in early childhood and is the first step in the allergic march preceding the development of respiratory allergic diseases, such as asthma and allergic rhinitis, later in life.^{2,3}

The major pathogenesis of AD is dysfunction of the skin intrinsic barrier and cutaneous inflammation by the innate and adaptive immune systems. Although filaggrin deficiency has recently been shown to be a major cause of skin barrier defects in Caucasian populations,⁴ other proteinase⁵ and lipid⁶ components are involved in the skin barrier defect. The skin barrier defect itself induces or interacts with immunological dysregulation, leading to cutaneous inflammation. Environmental factors, including microbiomes, are also involved in the pathogenesis of AD.^{7,8}

Recently, as diverse phenotypes and endotypes of asthma have been found, individualized treatment of asthma has been noted.^{9,10} Trials of asthma classification based on mechanism as well as clinical characteristics can lead to personalized

treatment suitable for a particular asthma phenotype with similar characteristics.^{11,12} Cluster analyses to define subgroups of asthma identify new phenotypes that are different from those of patients in other clusters and help to predict responses to particular therapies or develop novel therapeutic strategies and new drugs.^{13,14}

Atopic dermatitis should be considered a heterogeneous disease considering clinical features and complex pathogenesis, like asthma. Cluster analysis is needed to define the clinical phenotypes of AD composed of heterogeneous groups. Generally, AD has been classified into intrinsic and extrinsic AD on the basis of serum total immunoglobulin (Ig)E levels and/or sensitization.¹⁵ However, this classification is too simple to represent the diverse phenotypes of AD. Sensitization to *Malassezia*, *Staphylococcus aureus* or herpes simplex virus can play a role in differentiating subtypes of AD.^{16,17} Depending on the morphology or localization of the skin lesions, AD has also been classified into nummular eczema, atopic prurigo, hand eczema or juvenile palmar and plantar dermatitis.¹⁸ However, there is little information on cluster analysis of clinical phenotypes of AD in early childhood, when AD usually develops. In

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this study, we aimed to identify clinical phenotypes of AD in early childhood using cluster analysis and the clinical characteristics of each cluster.

METHODS

Subjects and study design

We enrolled 636 children under 3 years of age who were treated for AD at the pediatric allergy outpatient clinic of Asan Medical Center from November 2012 to December 2015. AD was diagnosed by a pediatric allergist on the basis of the criteria suggested by Hanifin and Rajka.¹⁹ Children were excluded from enrollment if they had systemic diseases other than allergy. The subjects underwent comprehensive evaluation; they were physical examined and interviewed to assess their Scoring AD (SCORAD) and investigated for clinical phenotype, including family history and comorbidities. Blood sampling was performed to evaluate eosinophil count, white blood cell count, C-reactive protein, and serum total and specific IgE levels. The data were collected at the time of diagnosis. The study was approved by the institutional review board of Asan Medical Center (no. 2015-0801) in conformity with the Declaration of Helsinki.

Clinical assessments

We collected demographic data, including age, sex and body mass index. The following clinical data were collected: family history of AD; presence of recurrent wheezing, which was defined as at least two wheezing episodes confirmed by pediatricians; severity of AD assessed by SCORAD, graded as mild (0–15), moderate (16–40) or severe (>40);²⁰ white blood cell count and eosinophil count in the peripheral blood; and C-reactive protein.

Measurement of atopy

We measured serum total and specific IgE levels using ImmunoCAP™ FEIA (Phadia, Uppsala, Sweden). All children were checked for allergic sensitization to six common food allergens (i.e. milk, egg, wheat, soy, peanut and fish) by measuring serum allergen-specific IgE. In addition, children aged 1 year or older were checked for allergic sensitization to two common inhalant allergens (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and 2–3-year-old children were checked for allergic sensitization to four common inhalant allergens (i.e. cat, dog, cockroach, *Alternaria*). The method of measuring sensitization to common allergens was based on several studies.^{21–23} Allergic sensitization was defined as the presence of one or more of the measured specific serum IgE levels (>0.35 kIU/L).

Transepidermal water loss

Transepidermal water loss (TEWL) measurements were performed with an Evaporimeter MPA6 (Courage + Khazaka, Cologne, Germany) by the same trained physician. TEWL was recorded on two different parts of the body, eczematous skin (lesion) and normal-appearing skin (non-lesion), by applying a probe to the exposed skin for approximately 15 s until the

measurement was recorded. The site where the parent thought the most severe lesion was located was chosen as the lesion, and the forearm was chosen as the non-lesion. All measurements were performed in the same room with a temperature of 22°C and a relative humidity of less than 50%. All other conditions were controlled according to published guidelines.²⁴ TEWL was measured only when it was confirmed that the emollient was not applied to the child's skin within 12 h before visiting our clinic. TEWL measurements were available for 296 patients.

Statistical analysis

All statistical analyses were performed with SPSS version 21.0 (SPSS Korea Data Solutions, Seoul, Korea). Uniform cluster analysis methodology with a two-step approach was applied to classify phenotypes of AD within the studied population. In the initial step, hierarchical cluster analysis using Ward's method was performed to generate a dendrogram for estimation of the number of likely clusters. This estimate was pre-specified in a *k*-means cluster analysis that was used as the principal clustering technique, similar to the elbow method. Variables for cluster modeling were chosen based on their characterization of the AD phenotype. Eleven variables (i.e. sex, age at onset, age at diagnosis, white blood cell count, blood eosinophil count, C-reactive protein, serum total IgE level, allergic sensitization to either food or inhalant allergens, medical history of recurrent wheezing and family history of AD) were used to characterize the AD clusters. Continuous variables were recorded as means $\pm$ standard deviation or mean (95% confidence interval [CI]) and were standardized using *z*-scores. Categorical variables were described as frequencies with percentages and standardized using 0 or 1. Continuous variables were log transformed to approximate a normal distribution where this was indicated. A between-clusters comparison was performed by ANOVA or the χ^2 -test. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Of the 636 children enrolled, 572 children were included for the cluster analysis. Sixty-four children were excluded from the analysis because of insufficient demographic data, clinical data or results of blood samples. The patients who underwent all blood tests except TEWL were included in the analysis. Three hundred and forty-one (59.6%) were boys and 231 (40.4%) were girls. The mean age of the children was 13.29 months and the standard deviation was 8.71 months. Other clinical characteristics of the subjects are presented in Table 1.

Cluster analysis

Using the hierarchical cluster analysis by Ward's method, a dendrogram that estimated the number of likely clusters was obtained and revealed four best-fit cluster models (Fig. 1). The four clusters were identified by *k*-means cluster analysis (Table 2). These clusters were distinguished in terms of sex, age at onset and diagnosis, white blood cell count, blood eosinophil count, C-reactive protein, serum total IgE level and

Table 1. Baseline characteristics of the study subjects

Characteristic	All patients (<i>n</i> = 572)
Male, <i>n</i> (%)	341 (59.6)
Age at diagnosis, months, mean $\pm$ SD	13.29 $\pm$ 8.71
Age at onset, months, mean $\pm$ SD	5.26 $\pm$ 3.39
Body mass index, kg/m ² , mean $\pm$ SD	17.05 $\pm$ 2.95
Family history of AD, <i>n</i> (%)	52 (9.1)
Recurrent wheezing, <i>n</i> (%)	16 (2.8)
WBC, / μ L, mean (95% CI)	9509 (7331–12 333)
Eosinophils, / μ L, mean (95% CI)	388 (164–502)
CRP, mg/dL, mean $\pm$ SD	0.17 $\pm$ 0.26
Serum total IgE, kU/L, mean (95% CI)	37.0 (8.7–157.6)
Food sensitization, <i>n</i> (%)	388 (67.0)
Inhalant sensitization, <i>n</i> (%)	50 (8.7)
Total SCORAD (mean $\pm$ SD)	23 $\pm$ 12
Objective SCORAD (mean $\pm$ SD)	15 $\pm$ 9
TEWL, lesion (<i>n</i> = 296) (mean $\pm$ SD)	50.4 $\pm$ 24.8
TEWL, non-lesion (<i>n</i> = 296) (mean $\pm$ SD)	19.3 $\pm$ 10.3

AD, atopic dermatitis; CI, confidence interval; CRP, C-reactive protein; IgE, immunoglobulin E; SCORAD, Scoring Atopic Dermatitis; SD, standard deviation; TEWL, transepidermal water loss; WBC, white blood cell.

sensitization to food or inhalant allergens. There were no significant differences in family history of AD or history of recurrent wheezing. The characteristics of each cluster are summarized in Table 2.

Cluster A

Cluster A had 141 members, of whom 97 (68.8%) were boys. Children in this cluster had early onset of AD (4.02 ± 2.22 months) and were diagnosed earlier (8.72 ± 5.51 months) than those in cluster D (8.69 ± 3.17 and 22.79 ± 6.74 months, respectively). Children in this cluster had higher blood eosinophil counts ($898/\mu\text{L}$; 95% CI, 478–1686) and white blood cell counts ($11\,849/\mu\text{L}$; 95% CI, 9509–14 765) than those in cluster B (eosinophils, $308/\mu\text{L}$ [95% CI, 162–584]; white blood cells, $8778/\mu\text{L}$ [95% CI, 7259–10 615]). They had higher serum total IgE levels (83.1 kU/L ; 95% CI, 23.8–290.0) and higher rates of sensitization to food allergens (123/141, 87.2%) than those in cluster B (total IgE, 12.7 kU/L

[95% CI, 4.4–36.2]; rate of allergic sensitization, 107/218 [49.1%]; respectively). Based on these characteristics, cluster A was termed “early-onset AD with high eosinophil and food sensitization”.

Cluster B

Cluster B had 218 members, 121 (55.5%) of whom were boys. They had early onset of AD (3.65 ± 2.11 months) and were diagnosed earlier (9.05 ± 5.51 months) than those in cluster D (8.69 ± 3.17 and 22.79 ± 6.74 months, respectively). Children in cluster B had lower serum total IgE levels (12.7 kU/L ; 95% CI, 4.4–36.2) than those in clusters A (83.1 kU/L ; 95% CI, 23.8–290.0) and D (71.5 kU/L ; 95% CI, 21.3–239.8). The rate of sensitization to food allergens in cluster B (107/218, 49.1%) was lower than those in clusters A (123/141, 87.2%) and D (111/160, 69.4%). The rate of sensitization to inhalant allergens in cluster B (7/218, 3.2%) was also lower than those in clusters A (9/141, 6.4%) and D (30/160, 18.8%). Cluster B was termed “early-onset, non-allergic AD”.

Cluster C

Cluster C had 53 members, 34 (64.2%) of whom were boys. Cluster C was the smallest group. Children in this cluster began to show symptoms of AD early (4.83 ± 3.47 months), as did children in cluster A (4.02 ± 2.22 months) and cluster B (3.65 ± 2.11 months). However, they were diagnosed later (14.21 ± 8.70 months) than children in the other two clusters with similar onset of AD (cluster A, 8.72 ± 5.51 months; cluster B, 9.05 ± 5.51 months). Children in cluster C were characterized by higher C-reactive protein ($0.77 \pm 0.59\text{ mg/dL}$) and white blood cell counts ($10\,097/\mu\text{L}$; 95% CI, 7631–13 360) than those in the other clusters. Cluster C was termed “early-onset AD with high C-reactive protein”.

Cluster D

Cluster D had 160 members, of whom 89 (55.6%) were boys. Children in this cluster had middle onset of AD and were diagnosed later than those in the other clusters. The mean age at onset and the mean age at diagnosis were 8.69 ± 3.17 and 22.79 ± 6.74 months, respectively. Children in this cluster had higher total IgE levels (71.5 kU/L ; 95% CI, 21.3–239.8) and rates of sensitization to inhalant allergens (30/160, 18.8%) than

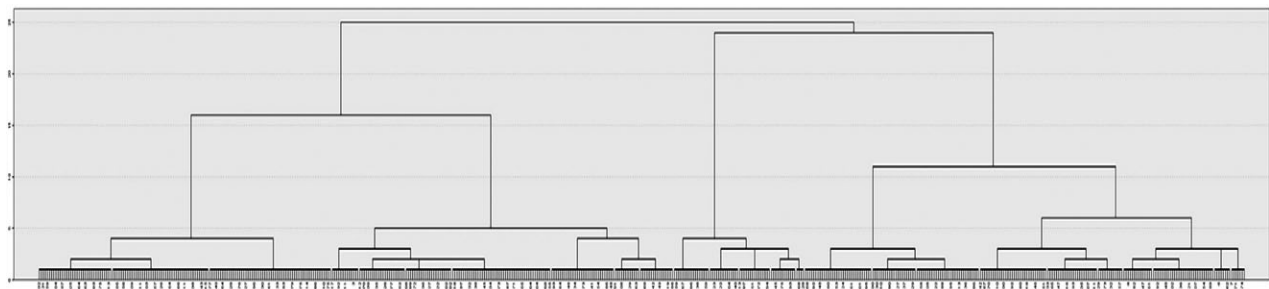
**Figure 1.** Dendrogram acquired by hierarchical cluster analysis by Ward's method.

Table 2. Characteristics of the children according to cluster analysis

Characteristic	Cluster A (<i>n</i> = 141)	Cluster B (<i>n</i> = 218)	Cluster C (<i>n</i> = 53)	Cluster D (<i>n</i> = 160)	<i>P</i>
Male, <i>n</i> (%)	97 (68.8) ^{>D}	121 (55.5)	34 (64.2) ^{>D}	89 (55.6) ^{<A,C}	0.047
Age at diagnosis (months, mean ± SD)	8.72 ± 5.51 ^{<C,D}	9.05 ± 5.51 ^{<C,D}	14.21 ± 8.70 ^{>A,B, <D}	22.79 ± 6.74 ^{>A,B,C}	<0.001
Age at onset (months, mean ± SD)	4.02 ± 2.22 ^{<D}	3.65 ± 2.11 ^{<D}	4.83 ± 3.47 ^{<D}	8.69 ± 3.17 ^{>A,B,C}	<0.001
Family history of AD, <i>n</i> (%)	14 (9.9)	17 (7.8)	4 (7.5)	17 (10.6)	0.760
Recurrent wheezing, <i>n</i> (%)	3 (2.1)	5 (2.3)	2 (3.8)	6 (3.8)	0.764
WBC (/ μ L, mean, 95% CI)	11 849 (9509–14 765) ^{>B,C,D}	8778 (7259–10 615) ^{<A,C}	10 097 (7631–13 360) ^{A<,>B,D}	8519 (6634–10 938) ^{<A,C}	<0.001
Eosinophils (/ μ L, mean, 95% CI)	898 (478–1686) ^{>B,C,D}	308 (162–584) ^{<A}	293 (98–880) ^{<A}	279 (137–567) ^{<A}	<0.001
CRP (mg/dL, mean ± SD)	0.12 ± 0.05 ^{<C}	0.11 ± 0.04 ^{<C}	0.77 ± 0.59 ^{>A,B,D}	0.11 ± 0.04 ^{<C}	<0.001
Serum total IgE (kU/L, mean, 95% CI)	83.1 (23.8–290.0) ^{>B}	12.7 (4.4–36.2) ^{<A,C,D}	48.9 (12.7–188.7) ^{>B}	71.5 (21.3–239.8) ^{>B}	<0.001
Food sensitization, <i>n</i> (%)	123 (87.2) ^{>B,D}	107 (49.1) ^{<A,C,D}	42 (79.2) ^{>B}	111 (69.4) ^{<A,>B}	<0.001
Inhalant sensitization, <i>n</i> (%)	9 (6.4) ^{<D}	7 (3.2) ^{<D}	4 (7.5)	30 (18.8) ^{>A,B}	<0.001

Superscript letters indicate groups that are significantly different at *P* < 0.05. AD, atopic dermatitis; CI, confidence interval; CRP, C-reactive protein; SD, standard deviation; WBC, white blood cells.

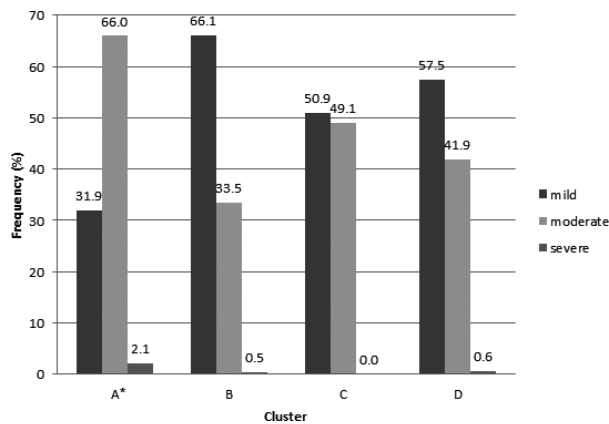


Figure 2. Frequency of children with mild, moderate and severe atopic dermatitis (AD) defined by objective SCORAD (Scoring AD) of each cluster. *When comparison between cluster A and other clusters by χ^2 -test, *P* < 0.05.

those in cluster B (IgE, 12.7 kU/L [95% CI, 4.4–36.2]; rate of allergic sensitization, 107/218 [49.1%]). Cluster D was termed “middle-onset AD with inhalant sensitization”.

Comparison of SCORAD and TEWL between clusters

We compared objective SCORAD values related to the severity of AD between clusters. Cluster A had the highest objective SCORAD of the four clusters (20 ± 10) (Table 3). Cluster A had a significantly lower percentage of subjects in the mild group (31.9%) than did the other clusters (cluster B, 66.1%; cluster C, 50.9%; cluster D, 57.5%), a significantly higher percentage in the moderate group (66.0%) than did the other clusters (cluster B, 33.5%; cluster C, 49.1%; cluster D, 41.9%) and a significantly higher percentage in the severe group (2.1%) than did the other clusters (cluster B, 0.5%; cluster C, 0.0%; cluster D, 0.6%) (Fig. 2).

We also compared skin barrier function, as measured by TEWL of the lesion, between clusters. Cluster A had the highest TEWL (59.2 ± 28.3), as it had the highest objective SCORAD. Clusters other than cluster A had similar TEWL values. The non-lesion TEWL values were similar in all four clusters.

Predictors of cluster assignment

Age at onset, age at diagnosis, white blood cell count, eosinophil count, C-reactive protein and serum total IgE level were identified as the strongest predictors of cluster assignment

Table 3. Objective SCORAD and TEWL scores of each cluster

Score	Cluster A	Cluster B	Cluster C	Cluster D	<i>P</i>
Objective SCORAD	20 ± 10 ^{>B,D} (<i>n</i> = 141)	13 ± 7 ^{<A,C} (<i>n</i> = 218)	17 ± 9 ^{>B} (<i>n</i> = 53)	15 ± 8.5 ^{<A} (<i>n</i> = 160)	<0.001
TEWL (lesion)	59.2 ± 28.3 ^{>B,D} (<i>n</i> = 78)	46.9 ± 23.0 ^{<A} (<i>n</i> = 102)	46.7 ± 19.8 (<i>n</i> = 24)	47.7 ± 23.3 ^{<A} (<i>n</i> = 90)	0.003
TEWL (non-lesion)	21.1 ± 11.7 (<i>n</i> = 78)	17.6 ± 7.7 (<i>n</i> = 103)	21.5 ± 9.2 (<i>n</i> = 24)	19.1 ± 11.7 (<i>n</i> = 90)	0.106

Superscript letters indicate groups that are significantly different at *P* < 0.05. SCORAD, Scoring Atopic Dermatitis; TEWL, transepidermal water loss.

(Wilks's $\lambda = 0.177$, $P < 0.001$). These six variables resulted in correct classification of 95.5% of the original studied population.

DISCUSSION

In this study, we identified four distinct phenotypes of AD in early childhood on the basis of clinical characteristics: early-onset AD with high eosinophil and food sensitization; early-onset, non-allergic AD; early-onset AD with high C-reactive protein; and middle-onset AD with inhalant sensitization. The key variables distinguishing the clusters were the ages at onset and diagnosis of AD, white blood cell count, blood eosinophil count, C-reactive protein and serum total IgE. Cluster analysis using these six variables resulted in four phenotypes with correct classification of 95.5% of the original studied population. There were differences between four in each cluster in terms of SCORAD and TEWL of the lesion.

Early-onset AD with high eosinophil and food sensitization (cluster A)

The subjects in this cluster had higher rates of food sensitization than those in the other clusters. Several studies have shown that food allergy is associated with AD.^{25–27} Furthermore, early-onset AD is known to be linked with sensitization to food allergens at 3 months of age.²⁸ Serum total IgE level was higher than that in other groups, as well as eosinophil counts. The level of serum total IgE increased with age.²⁹ This cluster showed high serum total IgE levels, although the subjects in this cluster were the youngest among the four clusters. This cluster had IgE-mediated immune responses, particularly to food allergens, resulting in eosinophilic inflammation. AD in children in this cluster seems to be similar to extrinsic AD in early childhood.

Early-onset, non-allergic AD (cluster B)

This cluster was the largest of the four clusters, with 37.8% of all subjects. Tokura *et al.*¹⁵ showed that some patients with AD had normal IgE levels for their age and lower rates of sensitization to allergens, although most AD patients had high serum total IgE levels.³⁰ This subtype of AD has been classified as “non-allergic”, “non-IgE-mediated” or “intrinsic” AD. Cluster B in our study was also designated as “non-allergic” AD. Non-allergic AD may have a different pathophysiology from extrinsic AD, with high serum total IgE levels and/or sensitization to allergens, although this has not yet been fully revealed. It is known that non-allergic AD symptoms subside over time and is less likely to develop into asthma.³¹ Therefore, it is necessary to follow up this cluster afterwards and check the progression of AD.

Early-onset AD with high C-reactive protein (cluster C)

This cluster was the smallest of the four clusters, with 9.3% of the subjects. Children in this cluster had higher C-reactive protein and white blood cell counts than those in other clusters. Unlike the children in cluster A who had a high eosinophil

count, it was thought that the high white blood cell count in cluster C was not caused by eosinophils. C-reactive protein and white blood cell count are typical infection markers^{32,33} that increase during infection, including in patients with AD.³⁴ Secondary skin infections, such as impetigo by *S. aureus* infection or eczema herpeticum by herpes simplex virus infection, are common in patients with AD.^{35,36} Considering the three points mentioned above, in this subtype of AD it can be assumed that there is a possibility of skin infection. Elevation of white blood cell count may be a secondary manifestation of infection. However, this interpretation may be compromised because we did not evaluate the presence of skin infection at enrollment.

Middle-onset AD with inhalant sensitization (cluster D)

Children in this cluster, with 28% of subjects, were associated with sensitization to inhalant allergens and had later onset of AD than those in the other clusters. The sensitization to inhalant allergens appeared after the age of 2 years²¹ and tended to increase with age.^{37,38} Children in this cluster had a lower food sensitization rate than those cluster A. Therefore, AD in children in this cluster seems to be associated with the sensitization to inhalant allergens regardless of sensitization to food allergens.

The severity of AD was expressed as SCORAD. The severity of AD was associated with sensitization to food at an early age^{38,39} and elevated allergen-specific IgE levels.⁴⁰ In our cluster analysis, this tendency was observed in cluster A, which had early onset of AD and a higher rate of sensitization to food allergens, although the number of patients with severe AD was so small in this study. Cluster B, which was characterized by non-allergy, had the lowest SCORAD.

It may be associated with SCORAD that the age of diagnosis in cluster A was younger than cluster C, with early-onset AD. Because the patients with high SCORAD visit a hospital earlier, as a result they can be diagnosed with AD more quickly. However, there are likely to be other factors besides SCORAD.

Like SCORAD, TEWL was higher in cluster A than in the other clusters. TEWL was considered to be a secondary result of skin inflammation, as indicated by the absence of differences between clusters in TEWL in non-lesions.

In one previous study published in 2015 to define AD phenotypes leading to asthma in infants younger than 1 year, AD was classified into three phenotypes.³⁸ They were “AD with low sensitization”, “AD with multiple sensitizations” and “AD with familial history of asthma”. These clusters differed by age, sex, SCORAD, parental history of asthma, food allergy and sensitization to food and inhalant allergens. In this ORCA study, the phenotype of AD with low sensitization was similar to that of our cluster B, early-onset, non-allergic AD. The phenotype of AD with multiple sensitizations was similar to that of our cluster A. The phenotype of AD with familial history of asthma was not correlated with our results. Although the results of our study may be different because of some differences in the purpose, subjects and methods from the ORCA

study, our study also showed that AD was classified into several clinical phenotypes.

Recently, Lavina *et al.*⁴¹ have published a study to identify distinct subgroups of AD in a longitudinal setting by using statistical methods other than cluster analysis. This study included genetic factors like filaggrin mutations as well as non-genetic factors. AD was classified into six subgroups including two of “persistent” groups. These two were strongly associated with the genetic mutations. Three of the six subgroups were early-onset AD groups with prevalence of AD before age of 30 months. They were reclassified according to timing of AD resolution. We did not include genetic mutations known to play a major role in skin barrier dysfunction of AD. It is necessary to check the association with genetic mutation and our clusters.

The main limitation of our study is that we have not identified changes in clinical features over time. AD is a chronic relapsing disease and varies with age, even in 3-year-old children.⁴² Therefore, it is necessary to study the disease course in patients in each cluster considering variation in the symptoms with age. The second limitation is that the rate of sensitization to inhalant allergens may be higher in children over 2 years old. In children 2 years of age, four more allergens (i.e. cat, dog, cockroach and *Alternaria*) were used to confirm sensitization to inhalant allergens than were used in children under 2 years of age. If more allergens are tested, the sensitization rate could be higher, because we presented a patient as sensitized if only one of the used allergens tested was positive. To correct for this effect, we performed cluster analysis using only two inhalant allergens (*D. pteronyssinus* and *D. farinae*), and the result was the same. In this study, children in clusters A, B and C were younger than in cluster D so that they did not receive inhalant allergen tests. Therefore, there is a possibility that the rate of sensitization to inhalant allergens in cluster D showed higher than the other clusters. The third limitation is that our results differ from those of previous cluster analyses of AD. This may be due to differences in the variables used in the cluster analysis, such as race, food allergy and environmental factors. Finally, a history of recurrent wheezing did not play a key role in distinguishing clusters from each other in our study although recurrent wheezing is known to be associated with high levels of serum total IgE and allergic sensitization.^{43,44} This was likely due to the fact that more than 50% of the children enrolled in our study were under 1 year old, and therefore they had not yet experienced two or more incidents of wheezing at the time of diagnosis of AD.

These results, obtained through cluster analysis, support the heterogeneity of AD, even in early childhood, and define more homogeneous subtypes of AD. The characteristics of these subtypes will help in the long-term management of AD. It may be that the colonization of *S. aureus* can be checked in group C and the occurrence of asthma or allergic rhinitis can be checked more carefully in group D than group B.

This study demonstrates that a four-cluster model was the best fit for children with AD under 3 years of age. Age at onset, age at diagnosis, white blood cell and eosinophil counts, C-reactive protein and serum total IgE level were the most

influential factors that helped distinguish each cluster. These results support the heterogeneity of AD, even in early childhood, and define more homogeneous subtypes of AD. Further studies using other clinical variables and longitudinal studies of each cluster are needed.

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CONFLICT OF INTEREST: None declared.

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