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Original Article

Fluctuations of aeroallergen-specific immunoglobulins and children's allergic profiles: Japan Environment & Children's Study of a pilot cohort

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CXCL7/NAP2, neutrophil activation factor 2;

DCP, densely carboxylated protein;

ISAAC, International Studies of Asthma and

Allergies in Childhood; JECS, Japan

Environment and Children's Study;

JPAC, Japanese Pediatric Asthma Control;

LIF, leukemia inhibitory factor; MIP-

1D, macrophage inflammatory protein-1D;

PCA, Principal component analysis;

POEM, Patient-Oriented Eczema Measure

ABSTRACT

Background: Allergen-specific immunoglobulins have a crucial role in allergic diseases. Most wheeze episodes develop before school age, and allergic rhinitis later develops during early elementary school years. However, the clinical background and cytokine/chemokine profiles associated with changes in immunoglobulins during early school-age are poorly understood.

Methods: This study used blood samples from children participating in the JECS Pilot Study. We examined nineteen kinds of aeroallergen-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) levels in patients at age 6 and age 8. Fluctuations of Der f 1- and Cry j 1-specific immunoglobulins levels during the two periods were compared to assess the frequency of allergic statuses and clusters of cytokine/chemokine profiles.

Results: The medians of aeroallergen-specific IgE levels did not fluctuate, and almost all IgG1 and IgG4 decreased. In IgA, four (e.g., Der f 1) increased, whereas the other four (e.g., Cry j 1) decreased. The ratio of the Der f 1-specific IgG1 level at age 8 to that at age 6 was higher in children with poor asthma control than in children with better asthma control. Moreover, the cytokine/chemokine cluster with relatively lower IL-33 and higher CXCL7/NAP2 was associated with lower Der f 1- and Cry j 1-specific IgG4 levels, but not IgE levels.

Conclusions: The cluster of cytokine/chemokine profiles characterized by lower IL-33 and higher CXCL7/NAP2 was associated with the maintenance of aeroallergen-specific IgG4 levels. This result provides a basis for considering the control of aeroallergen-specific immunoglobulins.

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Introduction

Allergen-specific IgE, which was discovered by Ishizaka *et al.* over 50 years ago, is a crucial factor involved in the development and exacerbation of allergic diseases.^{1–3} Allergen-specific IgE promotes the degranulation of mast cells by binding and cross-linking

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with the recognized allergen. This mechanism causes symptoms such as immediate allergic symptoms and anaphylaxis.^{4,5} Most allergic rhinitis and immediate-type food allergies depend on allergen-specific IgE levels.^{6,7} A high allergen-specific IgE level is associated with more severe symptoms in allergic diseases.^{8,9} It has been reported that a reduction of allergen-specific IgE level in allergic rhinitis is associated with an alleviation of allergic rhinitis symptoms.⁸ Another article demonstrated that in asthma, the allergen-specific IgE level was associated with wheezing and the severity of symptoms, independently from eosinophils in sputum.⁹ Based on these facts, allergen-specific IgE is an important target for the diagnosis and treatment of allergic diseases such as food allergy, allergic rhinitis, atopic dermatitis, bronchial asthma, and chronic urticaria.^{10,11}

Allergen-specific IgGs, particularly IgG4, are protective factors against the action of allergen-specific IgE.^{12,13} The ratio of the peanut-specific IgE level to the peanut-specific IgG4 level in peanut allergy is associated with the severity of allergic symptoms.¹⁰ Additionally, the allergen-specific IgG4 level increases during allergen immunotherapy,¹⁴ and in allergen immunotherapy for house dust mite, basophil activation is triggered when allergen-specific IgG4 was removed from the serum.¹⁵ Allergen-specific IgA has a protective role or preventive role against allergic diseases such as atopic dermatitis.¹⁶ Thus, additional mechanisms for the development and prognosis of allergic diseases can be revealed by examining the co-activities of immunoglobulins such as IgG subclasses and IgA, rather than examining specific IgE levels alone.

In addition, the levels of allergen-specific immunoglobulins and the type of allergens that are recognized by immunoglobulins change with age, even within the same individual. In general, the response to food allergens is remarkably higher during infancy and decreases thereafter. The response to the food allergens is remarkably higher first, but the response to aeroallergens becomes higher later.^{17,18} School environments, where children spend much of their time, are important for sensitization to aeroallergens.^{19,20} Initial exposure to the school environment when children are in lower grades of elementary school might change their aeroallergen-specific immunoglobulins levels. Moreover, this initial exposure period is when repertoires and somatic hypermutations of human B cell receptors such as IgG and IgA begin to increase toward their adult statuses, which many children reach at around the age of 10.^{21,22} These facts indicate that knowledge of immunological statuses associated with fluctuations of aeroallergen-specific immunoglobulins levels during early school age contribute to the prediction of aeroallergen-specific immunoglobulins levels during a subsequent period. Our previous study focusing on food-associated allergen-specific immunoglobulins levels during the early infantile stage did not reveal fluctuations of aeroallergen-specific immunoglobulins during early school age.²³ Furthermore, most wheezing episodes develop before school age, whereas the occurrence of allergic rhinitis rapidly increased during early elementary school years, according to a birth cohort followed in Tokyo.^{24,25} The age of early-school age is the turning point for airway allergic symptoms. Therefore, we consider that age of 6 and 8 years is a critical time point to evaluate immunological changes. Therefore, we conducted this study by focusing on data of patients from age 6 to age 8 to identify associations with fluctuations of aeroallergen-specific immunoglobulins levels.

The presence of allergic diseases is associated with developing sensitization to allergens.^{17,23} Although this indicates that the current allergic status may affect later fluctuations of allergen-specific immunoglobulins levels (including IgG subclasses and IgA) during early school age, it is not clear whether the same factors are

important in other periods, such as infancy. In addition to the allergic profile, fluctuation of immunoglobulin levels is another relevant factor. Cytokines are associated with changes in allergen-specific IgE in B cells. Some cytokines, such as IL-5 and IL-21, stimulate IgE production in B cells in the mice and humans at the cellular level and are associated with allergic sensitizations.^{26,27} However, many types of cytokines and chemokines are linked to and dependent upon each other. Therefore, it is difficult to grasp cytokines as a single action clinically. We previously reported that the cytokine profiles of 6-year-old children from this cohort can be distinguished into three clusters.²⁸ This study classified three clusters based on cytokine/chemokine. Cluster 1 had slightly higher levels of leukemia inhibitory factor [LIF] and lymphotactin than other clusters. Cluster 2 had higher levels of IL-33, IL-9, CCL3/macrophage inflammatory protein-1D [MIP-1D], and IL-17A than other clusters. Cluster 3 had relatively higher CXCL7/NAP2 [neutrophil activation factor 2] than other clusters. Asthma-related symptoms were mainly found in clusters 2 and 3, but not in cluster 1. These cytokine profiles might affect allergen-specific immunoglobulin levels. However, few reports comprehensively examine the association between fluctuations in allergen-specific immunoglobulin (IgE, IgGs, and IgA) levels and allergic statuses and cytokines.

As mentioned above, the contribution of allergen-specific immunoglobulins (IgG1, IgG4, IgA, and IgE) to allergic diseases is considerable. These changes in allergen-specific immunoglobulin levels overlap with the timing of the development of airway allergies such as asthma and allergic rhinitis.²⁹ Identifying changes in allergen sensitization from preschool to elementary school will lead to a better understanding of the clinical background. With sufficient knowledge about the association between allergic statuses, cytokine/chemokine profiles, and fluctuations of allergen-specific immunoglobulins, it will be possible to contribute to the strategy of capturing the allergic march. Herein, we examined whether allergic statuses and cytokine/chemokine profiles were associated with fluctuations of aeroallergen-specific immunoglobulin levels in children from 6 years to 8 years of age.

Methods

Study design, setting, and participants

The study design was an exploratory cohort study of children ages 6 and age 8 that utilized data from a cohort study. This study used blood samples from children participating in a pilot birth cohort study of the Japan Environment and Children's Study (JECS).^{28,30,31} The JECS Pilot Study is still in progress at four different centers (Jichi Medical University [Tochigi prefecture], University of Kyusyu [Fukuoka prefecture], University of Occupational and Environmental Health [Fukuoka prefecture], and University of Kumamoto [Kumamoto prefecture]) in Japan. Between February 2009 and March 2010, the Pilot Study was explained to include pregnant women receiving outpatient care from cooperating local healthcare providers of the regional centers participating in the JECS Pilot Study and their partners. Informed consent for participation was provided by 453 pregnant women (Supplementary Fig. 1). Among them, 440 children were born from 453 mothers who participated in the JECS Pilot Study. In the JECS Pilot Study, children of the leading group are now 12 years old. This study enrolled all children whose evaluable samples for allergen-specific immunoglobulins were obtained at ages 6 and 8 from children participating in the JECS Pilot Study. The study protocol was reviewed and approved by each regional center. Written consent was obtained from all caregivers of children before participation in the study.

Variables

The presence of ever/current asthma, ever/current eczema, and ever/current rhinitis at age 6 were confirmed by using a parent-reported questionnaire, ISAAC questionnaire (International Study of Asthma and Allergies in Childhood).^{32,33} The asthma control level was assessed in accordance with the Japanese Pediatric Asthma Control (JPAC) program.³⁴ The score scale ranged from 0 to 15, with 0–11, 12–14, 15 categorized as loss of control, almost control, and perfect control, respectively. A higher score indicates better control of asthma. The Patient-Oriented Eczema Measure (POEM) score was used to assess the level of eczema control. The number of children with severe eczema was lower than those with moderate eczema in this study.³⁵ Therefore, to perform statistical analysis, we categorized them into three categories: clear/almost clear, mild and moderate, and severe (moderate/severe/very severe). Information on the food allergic reaction history, pet ownership, environmental smoking, and parental allergic histories were obtained via the parent-reported questionnaire when the children were 6 years of age.

Measurement of cytokine/chemokine profiles

We used the results of clustering cytokine/chemokine using blood samples at age 6 years, as reported in our previous articles for this cohort study.²⁸ The plasma levels of cytokines/chemokines were measured using MultiPlex™ Human Cytokine/Chemokine Assay Kits (MILLIPLEX: Merck Millipore, Darmstadt, Germany). Moreover, principal component analysis (PCA) for all results of cytokines/chemokines was conducted by R, and three clusters of cytokines/chemokines with different characteristics were distinguished. We performed statistical analysis using these three clusters.

Measurement of aeroallergen-specific IgE, IgG1, IgG4, and IgA levels

Aeroallergen-specific IgE, IgG1, IgG4, and IgA levels of blood samples at ages 6 and 8 were measured using the Densely Carboxylated Protein (DCP) Microarray (AMERIC, Tokushima, Japan), which has a correlation of allergen-specific immunoglobulin levels with UniCAP system (Phadia AB)³⁶ and reported usefulness in food allergies,³⁷ and indicated the BU/ml level, as described previously.^{23,38} In this study, 19 aeroallergen-specific immunoglobulins (house dust [*Dermatophagoides farinae*; Der f 1], *Dermatophagoides pteronyssinus* [Der p 1], dog dander, cat dander, *Alternaria alternata* [Alt a 1], Asp f 1, Cla h 8, Japanese cedar, Cry j 1, Japanese cypress, birch, alder, Timothy grass, sweet vernal grass, orchard grass and Ragweed) were measured. The measurement limit value of each aeroallergen-specific IgE, IgG1, IgG4, and IgA was 0.01 BUe/mL, 100.0 BUg1/mL, 50.0 BUg4/mL, and 2.5 BUa/mL, respectively, under the present assay condition. In all statistical analyses of this study, each aeroallergen-specific immunoglobulin level under these limit values was used as the equivalent to the limit values. Moreover, aeroallergen-specific immunoglobulin levels with log-converted were used.

Bias and study size

To minimize bias, we included all children for whom data on aeroallergen-specific immunoglobulins were obtained from blood samples at both 6 and 8 years of age. Since this was an exploratory and hypothesis-generating study, we did not predefine a sample size for this study.

Statistical analysis for individually aeroallergen-specific immunoglobulins

To confirm the state of fluctuations in aeroallergen-specific immunoglobulins during the observed period, we compared each aeroallergen-specific immunoglobulin at age 6 and at age 8 by using the Wilcoxon signed-rank test. In comparing these tests, *P* values less than 0.00066 (0.05/76) were recognized as significant, as 76 statistical tests were conducted simultaneously. Next, we focused on the most popular components of aeroallergens Der f 1 and Cry j 1.²⁵ Ratios of these Der f 1 and Cry j 1-specific immunoglobulin levels at age 8 to those at age 6 were calculated using aeroallergen-specific immunoglobulin levels at age 6 as the denominator and represented as the fold. Associations between these folds, indicating fluctuations of Der f 1-specific and Cry j 1-specific immunoglobulin levels from age 6 to age 8, and the allergic status (presence of current wheeze, eczema, and rhinitis, as well as the control of asthma and eczema) at age 6 were analyzed using the Mann–Whitney *U* test (when between 2 groups) or Kruskal–Wallis test, followed by Dunn–Bonferroni tests (when performing a comparison among three groups). These folds were compared among clusters of cytokine/chemokine profiles at age 6 by using the Kruskal–Wallis test, followed by Dunn–Bonferroni tests. In comparing these tests, *P* values under 0.05 were recognized as significant. All statistical analyses for individually aeroallergen-specific immunoglobulins levels and comparisons using the results of this analysis were performed using the Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corporation, Armonk, NY, USA).

Table 1
Characteristics of children in this study.

	Number [†]	
Sex, male, n (%)	124	64 (51.6%)
Age, median (first quartile – third quartile)	124	72 (71–73)
Parental allergic history		
Mother, n (%)	121	39 (31.7%)
Father, n (%)	104	28 (26.9%)
Environmental smoking at 6 y, n (%)	115	42 (36.5%)
Pet ownership at 6 y		
Cat, n (%)	122	14 (11.5%)
Dog, n (%)	122	6 (4.9%)
Allergic history at 6 y		
Ever wheeze, n (%)	122	40 (32.8%)
Current wheeze, n (%)	122	21 (17.2%)
JPAC (first quartile – third quartile)	120	15 (15–15)
Perfect control, n (%)		93 (77.5%)
Good control, n (%)		22 (18.3%)
Poor control, n (%)		5 (4.2%)
Ever eczema, n (%)	123	26 (21.1%)
Current eczema, n (%)	123	25 (20.3%)
POEM (first quartile – third quartile)	123	0 (0–0)
Clear/almost clear, n (%)		97 (78.9%)
Mild, n (%)		16 (13.0%)
Moderate/severe/very severe, n (%)		10 (8.1%)
Ever rhinitis, n (%)	123	48 (39.0%)
Current rhinitis, n (%)	123	43 (35.0%)
Total IgE level (IU/mL) at 6 y	119	84.5 (19–257.5)
Median (first quartile – third quartile)		
Clusters of cytokine/chemokine profiles at 6 y ²⁸		
The cluster 1, n (%)		33 (28.6%)
The cluster 2, n (%)		41 (35.7%)
The cluster 3, n (%)		41 (35.7%)

JPAC, Japanese Pediatric Asthma Control program; POEM, Patient Oriented Eczema Measure; 6 y, 6 years old.

Reproduced from Reference.²⁸

[†] Number of children without a missing value.

Results

Participants

In this study, 124 children of 440 children who had participated in the JECS Pilot Study were included, of whom 64 children were boys (51.6%), and 83 children (66.9%) had an ever allergic history (asthma, eczema, rhinitis, or food allergy) (Table 1). In the control test of allergic diseases, 93 children (77.5%) occupied the category of “perfect control” in the JPAC asthma test, and 97 children (78.9%) occupied the category of “clear/almost clear” in POEM. Cytokine/chemokine clusters classified 115 children into three clusters: 33 children were classified as cluster 1, 41 as cluster 2 and 41 as cluster 3.²⁸

Fluctuations of each aeroallergen-specific immunoglobulin level

Figure 1 shows the results of comparing the median of each aeroallergen-specific immunoglobulin level between those at age 6 and those at age 8. Medians of all aeroallergen-specific IgE levels were observed under valuable levels, and there was no change between both ages. In aeroallergen-specific IgG1 levels, 15 of 19 aeroallergen-specific IgG1 levels at age 8 were lower than those at 6 years (except for IgG1 levels that were specific to house dust, *D. farinae*, Der f 1, and Cla h 8). All aeroallergen-specific IgG4 levels at age 8 were lower than those at age 6. Four (dog dander, Asp f 1, Cry j 1, and alder) of 19 aeroallergen-specific IgA levels at age 8 were lower than those at age 6. On the other hand, four (Der f 1, Cla

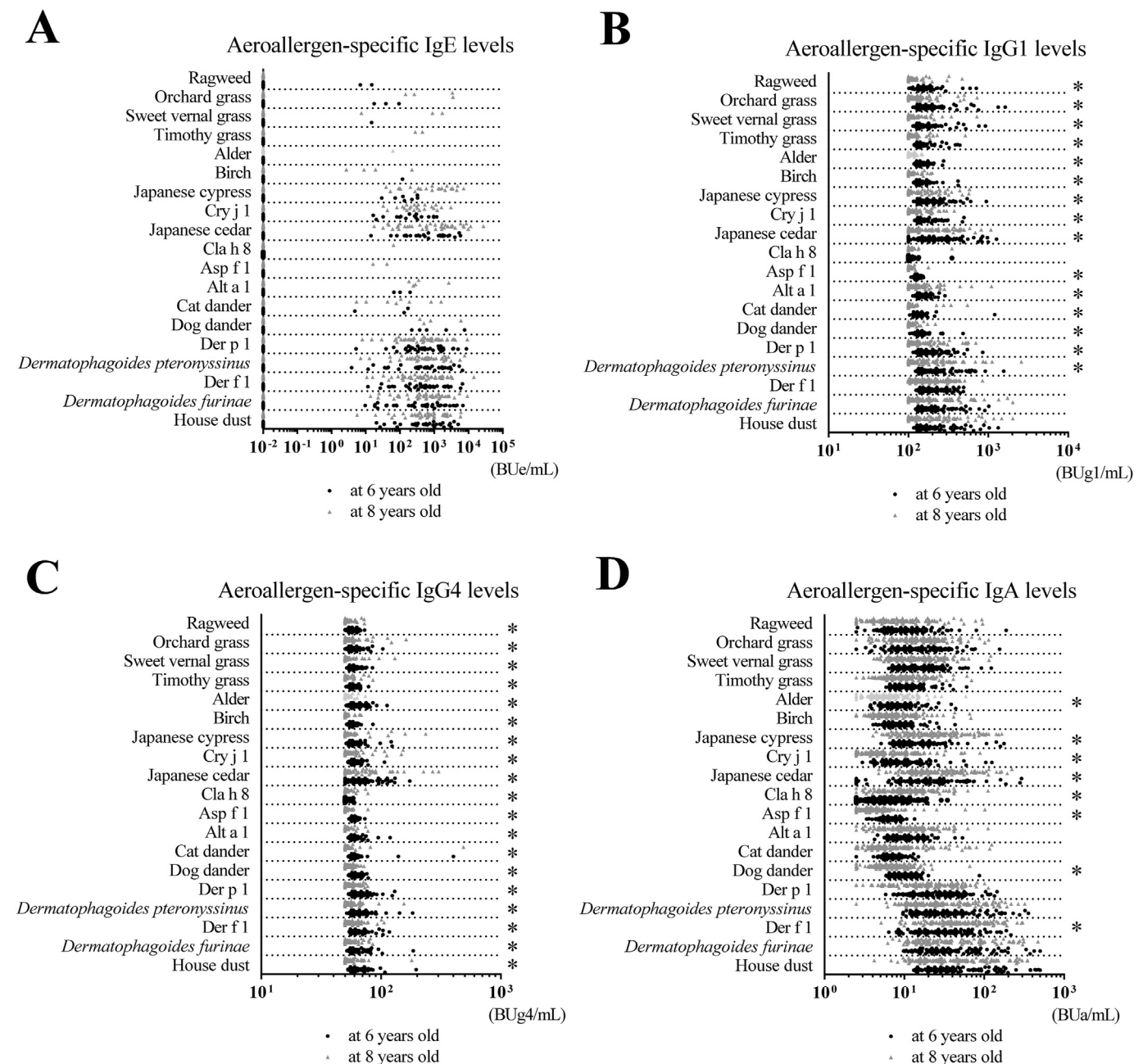


Fig. 1. Comparison between age 6 and age 8 years for each aeroallergen-specific immunoglobulin (IgE, IgG1, IgG4, and IgA) using the Wilcoxon signed-rank test. *P value < 0.00066 represents a significant difference.

h 8, Japanese cedar, and Japanese cypress) aeroallergen-specific IgA levels at age 8 were higher than at age 6.

Association between fluctuations of Der f 1- and Cry j 1-specific immunoglobulin levels from age 6 to age 8 and the presence of allergic diseases

Before we examined the associated fluctuations of aeroallergen-specific immunoglobulins from age 6 to age 8 and the allergic status, we selected representative allergens to avoid too many statistical tests. IgEs specific to house dust mite and Japanese cedar are common aeroallergens causing allergic rhinitis in Japan,^{25,38} and, in the above results, relatively many children participating in this study had measurable associated aeroallergen-specific immunoglobulin levels. Moreover, we were concerned that crude aeroallergen-specific immunoglobulins might include components with different changing patterns. We selected and focused on Der f 1, which is a major component of *D. farinae*, and Cry j 1, which is a major component of Japanese cedar. We conducted statistical analysis to compare the folds of Der f 1- and Cry j 1-specific immunoglobulin levels between children with current wheeze, eczema, and rhinitis and children without each allergic disease at age 6. Significant differences were not observed in comparing all folds of Der f 1- and Cry j 1-specific immunoglobulin levels between children with each allergic disease and without those at age 6 (Supplementary Fig. 2–4).

Association between fluctuations of Der f 1- and Cry j 1-specific immunoglobulin levels from age 6 to age 8 and control of allergic diseases

In considering the allergic status, the presence of allergic diseases and their control status are important. In the control of allergic diseases, among categories in JPAC at age 6, significant differences were shown in the fluctuation of the Der f 1-specific IgG1 level from age 6 to age 8. Children classified with poor control in JPAC had a higher-fold Der f 1-specific IgG1 level than children classified as having perfect control or good control (poor control vs. perfect control: 1.26 [1.02–1.98] vs. 0.83 [0.71–1.06], *P*-value: 0.045; poor control vs. good control: 1.26 [1.02–1.98] vs. 0.84 [0.64–0.96], *P*-value: 0.024) (Fig. 2). Among categories in POEM at age 6, there were no significant differences in the fluctuation of Der f 1- or Cry j 1-specific immunoglobulin levels from age 6 to age 8 (Fig. 3).

Association between fluctuations of Der f 1- and Cry j 1-specific immunoglobulin levels from age 6 to age 8 and cytokine/chemokine profiles

In our previous article, children were classified into three clusters on the basis of the result of PCA using cytokines/chemokines data at age 6 of the same children as this study.²⁸ Cytokines/chemokines build a network with each other. Therefore, three clusters which were shown in the previous article were used for valuing associations between cytokine/chemokine profiles and fluctuations of Der f 1- and Cry j 1-specific immunoglobulin levels, but not individual results of individual chemokines/cytokines. There were not significant differences among three cytokine/chemokine clusters in Der f 1- and Cry j 1-specific IgE levels. The children in cluster 3 did not have higher folds of aeroallergen-specific IgE levels compared to other clusters, but in Der f 1-specific immunoglobulin, children in cluster 3 had a higher-fold Der f 1-specific IgG4 level than children in cluster 1 and children in cluster 2 (cluster 3 vs. cluster 1: 0.90 [0.86–0.98] vs. 0.82 [0.78–0.84], *P*-value: <0.001; cluster 3 vs. cluster 2: vs. 0.83 [0.81–0.90], *P*-value: 0.005) (Fig. 4).

In Cry j 1-specific immunoglobulins, children in cluster 3 had a higher-fold Cry j 1-specific IgG4 level than children in cluster 2 (cluster 3 vs. cluster 2: 0.90 [0.84–0.96] vs. 0.83 [0.8–0.89], *P*-value: <0.001) (Fig. 4). Children in cluster 3 had a higher-fold Cry j 1-specific IgG1 level than children in cluster 1 (cluster 3 vs. cluster 1: 0.78 [0.70–0.95] vs. 0.72 [0.70–0.74], *P*-value: 0.020) (Fig. 4).

Discussion

We showed fluctuations of 19 aeroallergen-specific IgE, IgG1, IgG4, and IgA levels from age 6 to age 8. In all aeroallergen-specific IgE levels, there were no significant differences between those at age 6 and those at age 8. In 15 aeroallergen-specific IgG1 levels and all 19 aeroallergen-specific IgG4 levels, those at age 8 were lower than those at age 6. In four specific IgA levels against aeroallergens, those at age 8 were higher than those at age 6. When Der f 1 and Cry j 1 of these aeroallergens were focused, poor asthma control was associated with a higher-fold Der f 1-specific IgG1 level from age 6 to age 8 than that of children with better asthma control. Cluster 3 of cytokine/chemokine, characterized by a higher CXCL7/NAP, was associated with higher-fold Der f 1- and Cry j 1-specific IgG4 levels from age 6 to age 8.

This study did not show differences in all medians of aeroallergen-specific IgE levels between those at age 6 and those at age 8, and almost medians of aeroallergen-specific IgG1 and IgG4 levels at age 6 were higher than those at age 8. A previous article from Europe showed that geometric means of aeroallergen-specific IgG levels reached a peak level until children who participated in their birth cohort reached age 6.³⁹ At the same time, their cohort showed that aeroallergen-specific IgG levels were higher in children with allergic diseases than in children without those.³⁹ In another study, which included children with allergic diseases, children with sensitization to certain aeroallergens had higher specific IgG4 levels against the aeroallergen than children without sensitization aeroallergens.⁴⁰ However, many aeroallergen-specific IgG4 levels had a below-standard allergen-specific-IgG4 level, despite that study including only children with allergic diseases. Therefore, declines of some aeroallergen-specific immunoglobulin levels shown in our study might be associated with the fact that the number of children with allergic diseases was relatively fewer than those in these studies and increasing specific IgE levels from age 6 to age 8 had not been observed.

Although the presence of each allergic disease was not associated with the fluctuation of Der f 1- and Cry j 1-specific immunoglobulin levels, poor asthma control by JPAC at age 6 was associated with a higher-fold Der f 1-specific IgG1 level from age 6 to age 8. Guiddir *et al.* identified three clusters of children with severe recurrent wheeze and severe asthma during childhood.⁴¹ Children in an age group close to the age group of our study who were classified into two clusters had lower eosinophil counts in blood and bronchoalveolar lavage compared to clusters containing older children. These were consistent in that the poor control in JPAC in our previous report was not associated with increasing TH2 cytokines but was associated with CXCL7/NAP2, which is an activation factor of neutrophils. Neutrophil produces factors that have a crucial role in the survival, maturation, and differentiation of human B cells.⁴² These factors might explain why the mechanism of poor asthma control at age 6 was associated with a higher-fold Der f 1-specific IgG1 level from age 6 to age 8.

Folds of aeroallergen-specific IgE levels from age 6 to age 8 did not significantly differ among cytokine/chemokine profile clusters. Cluster 3 also had features, such as relatively higher CXCL7/NAP2, and cluster 3 had lower IL-33 than cluster 2.²⁸ The discussed cohort showed that an increasing Bet v 1 (a major component of birches)-specific IgG level subsequently induced an increase in the Bet v 1-

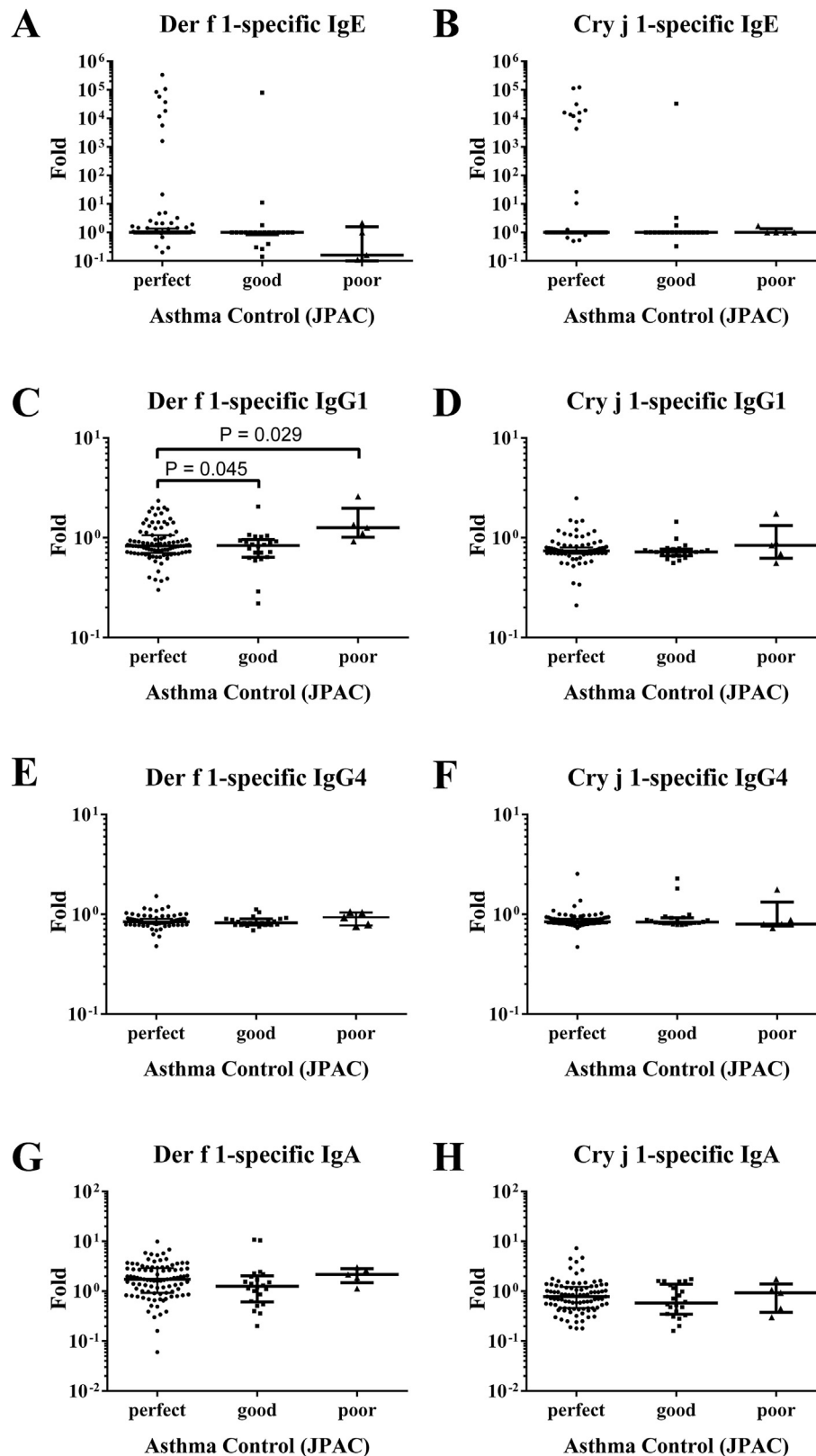


Fig. 2. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to age 8 and asthma control at age 6. Comparison between children among asthma controls by the Japanese Pediatric Asthma Control (JPAC) program at age 6 for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from ages 6–8 using the Kruskal–Wallis test followed by the Dunn–Bonferroni tests (A to H). The long bar at the center of the line that runs through the center of each dot means median and short bars above and below it mean the interquartile range. P value < 0.05 represents a significant difference.

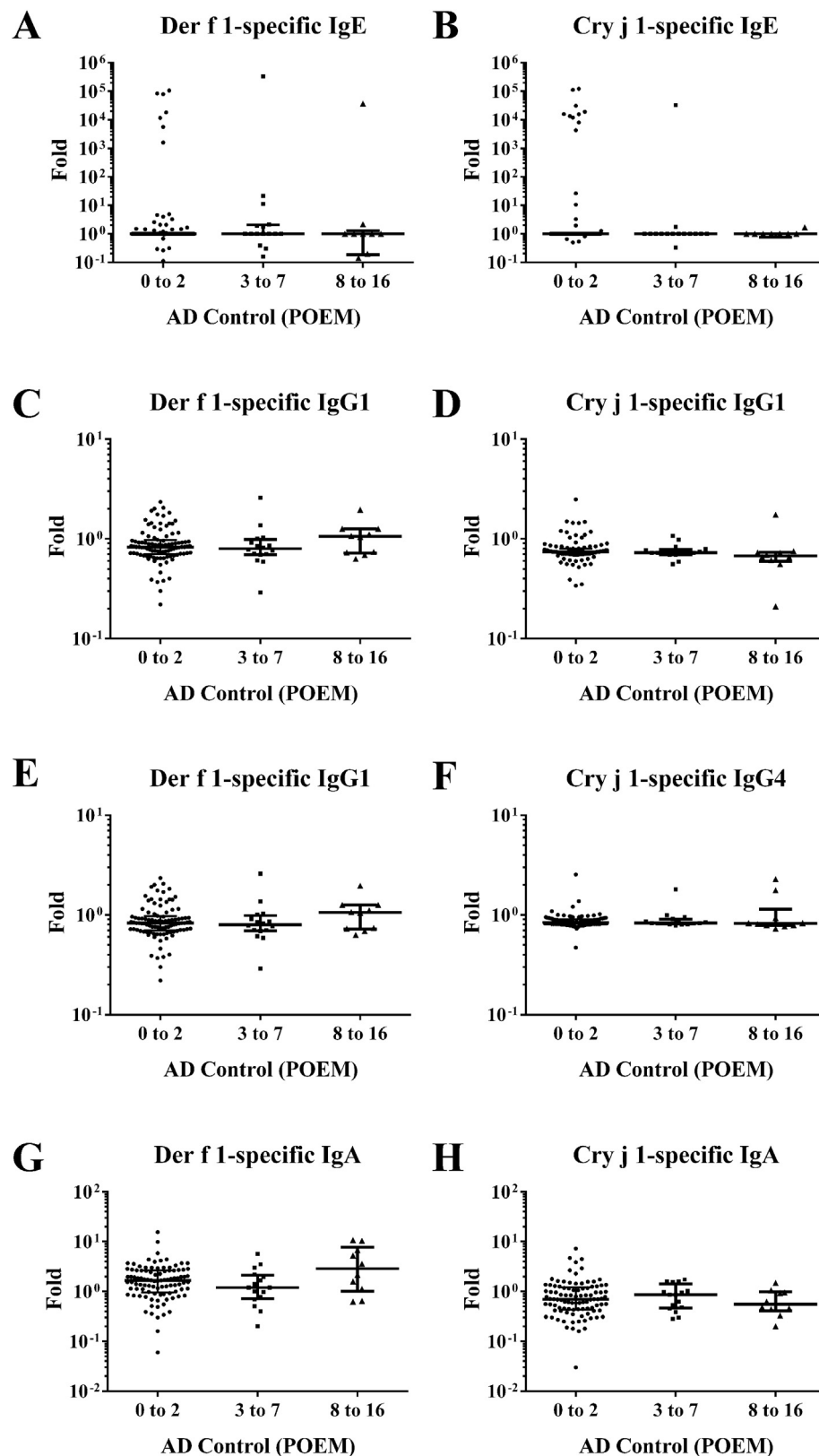


Fig. 3. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to age 8 and atopic dermatitis control at age 6. Comparison between children among atopic dermatitis controls by Patient-Oriented Eczema Measure (POEM) at age 6 for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from age 6 to age 8 using the Kruskal–Wallis test followed by the Dann–Bonferroni tests (A to H). The long bar at the center of the line that runs through the center of each dot means median and short bars above and below it mean the interquartile range. P value < 0.05 represents a significant difference.

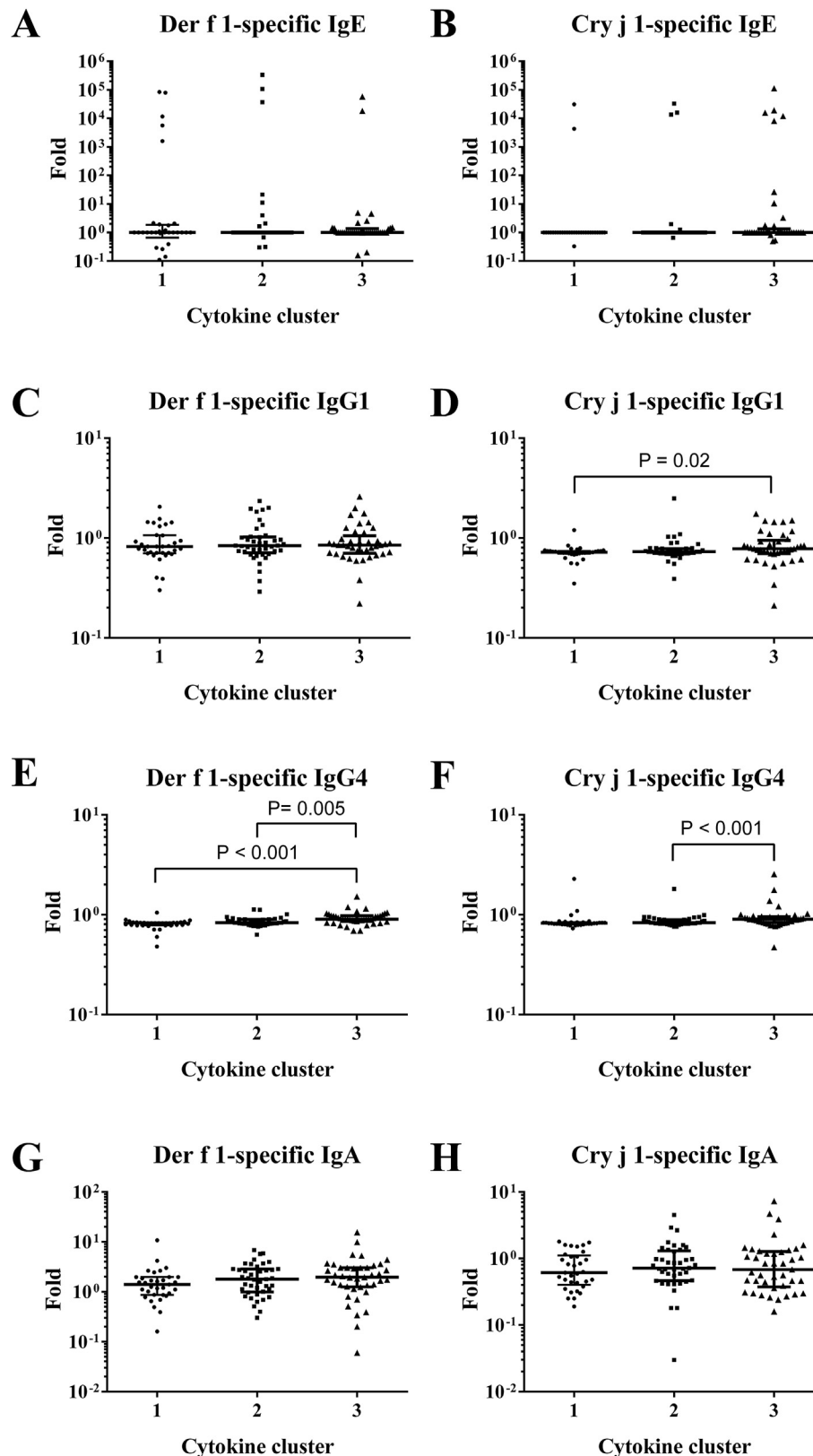


Fig. 4. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to age 8 and cytokine/chemokine profiles at age 6. Comparison between children among clusters of cytokine/chemokine profiles at age 6 for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from age 6 to age 8 using the Kruskal–Wallis test followed by the Dann–Bonferroni tests (A to H). The long bar at the center of the line that runs through the center of each dot means median and short bars above and below it mean the interquartile range. P value < 0.05 represents a significant difference.

specific IgE level in almost all cases.³⁹ There is a contradiction in the role of CXCL7/NAP2 when considering the results of our cohort with the results of the JPAC control and cytokines mentioned above, but it may be important to consider including other cytokines, such as IL-33, which is secreted from the neutrophil-activated airway epithelium upon contact with allergens and subsequently induces eosinophilic inflammation in the airway epithelium.⁴³ This eosinophilic inflammation facilitates allergic sensitization. Therefore, these facts suggest that suppressing IL-33 secretion under elevated CXCL7/NAP2 leads to the suppression of aeroallergen-specific IgE production.

Children in cluster 3 of cytokine/chemokine profiles had a higher-fold Der f 1-specific IgG4 level from age 6 to age 8 in comparison with other clusters and those of Cry j 1-specific IgG4 compared to those of cluster 2. Previously, the article showed that the allergen-specific IgG4 level was increased by allergen immunotherapy,¹⁵ and a lower IgE/IgG4 ratio may be associated with fewer allergic reactions.^{13,14} This means that the background, as cluster 3 of cytokine/chemokine was associated with an increasing aeroallergen-specific IgG4 level, might lead to a decrease in allergic reaction. Moreover, children in cluster 3 did not have higher folds of aeroallergen-specific IgE levels compared to other clusters. Therefore, these facts indicated that maintaining the cytokine profile, such as that of cluster 3 with a relatively higher CXCL7/NAP2 [neutrophil activation factor 2] than other clusters might improve immunotherapy. It has been reported that CXCL7/NAP2 is induced by endotoxin.⁴⁴ Another pilot study indicated that children on the farm showed a relatively higher immunotherapy efficacy than children in the urban area.⁴⁵ Authors in that study speculated that result was due to higher endotoxin levels in the farm area than those in the urban. These results might support our result. Comprehensive studies of aeroallergen-specific IgG1, IgE, and IgG4 and cytokine profiles are needed to confirm aforementioned hypothesis.

Limitations

First, one limitation was that Der f 1 and Cry j 1 were the selected aeroallergens for many of the analyses. Therefore, in other allergens except for Der f 1 and Cry j 1, we did not clarify what fluctuations there were. Therefore, future studies are needed to clarify those fluctuations. Second, this study did not have information about Der f 1 and Cry j1 exposure in regions where the children lived. However, a previous article showed only small differences in ratios of sensitization to house dust mites and Japanese cedar in pediatric patients among prefectures except for Hokkaido and Okinawa.⁴⁶ This article did not include children in Hokkaido and Okinawa. Therefore, the difference in the association between clinical allergic status and fluctuations of aeroallergen-specific immunoglobulin levels might also be small among prefectures where we conducted our research. Third, this study evaluated only the school aged children although we consider that age of 6 and 8 years is the critical time point to evaluate immunological changes since allergic rhinitis rapidly increases during early elementary school years and early-school age was the important turning point of airway allergy symptoms. Some articles stated that even children with the same allergic diseases or healthy children have different molecular features among different age groups.^{47,48} Therefore, results might be different from the studies conducted on different age groups, and detailed research is needed in each age group in a long-term future study. Fourth, these results might be different from studies that focus only on children who had already developed an allergic disease such as hospital-based medical chart data. However, we consider that our study design was of higher evidence level since we collected the data minimizing sampling bias and

general populations compared to a retrospective cohort study of medical chart data. This study could include both the general population and non-allergic children and allergic children.

Conclusion

Some allergic and cytokine/chemokine backgrounds were associated with immunoglobulin fluctuations against Der f 1 and Cry j 1. Clusters that had features such as higher CXCL7/NAP2 and lower IL-33 were associated with a narrower decrease of Der f 1- and Cry j 1-specific IgG4, not IgE. Although these results need to be confirmed with more extensive samples, they provide a new perspective on fluctuations of immunoglobulins and provide guidance for the treatment and prognosis prediction of allergic diseases.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2022.01.002>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

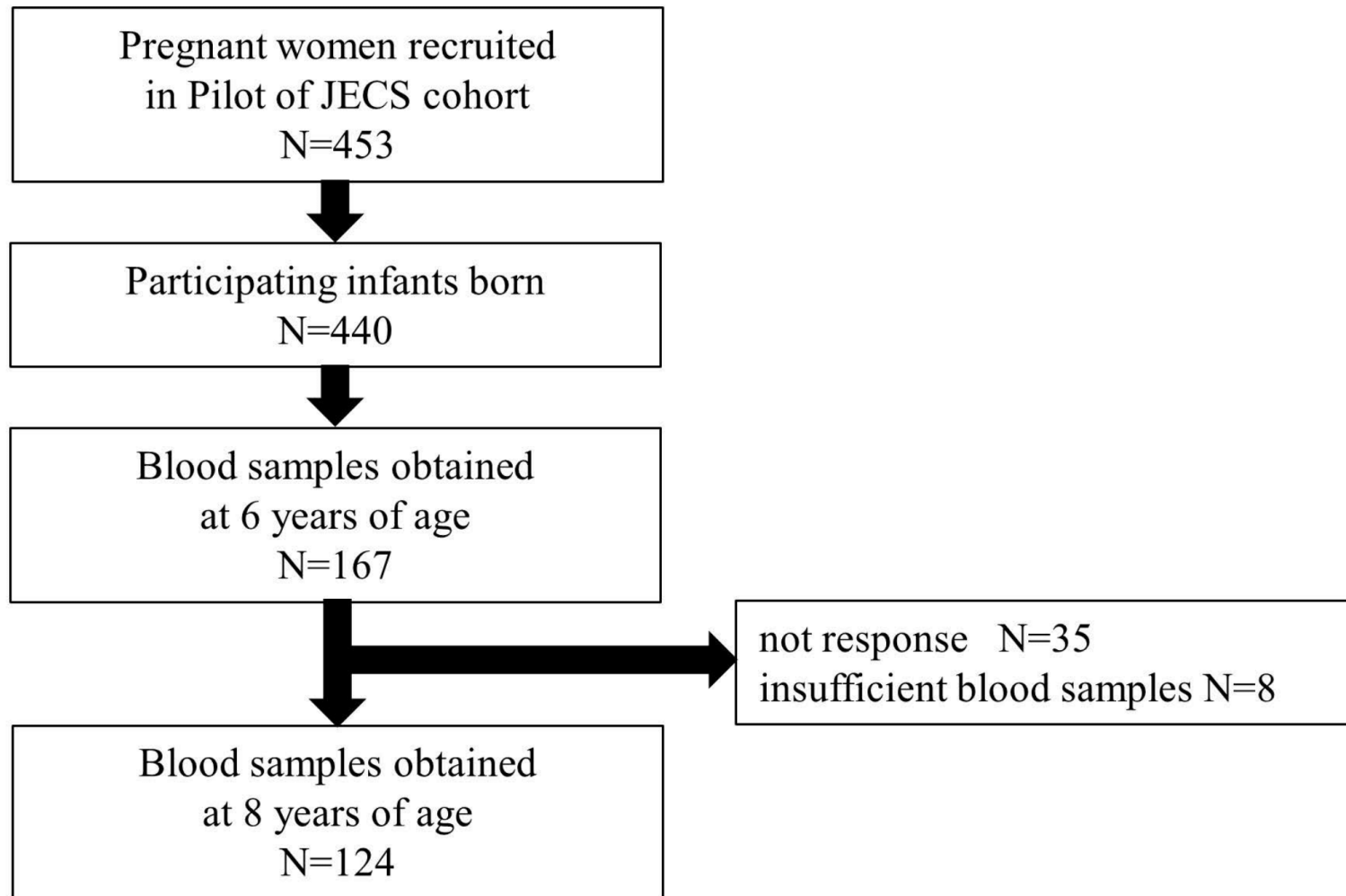
MI, KYH, MaS, MiS, YM, and YO were involved in the study design. All authors contributed to data interpretation. MI and LY were involved in the data analyses. All authors critically revised the report, commented on drafts of the manuscript, and approved the final report.

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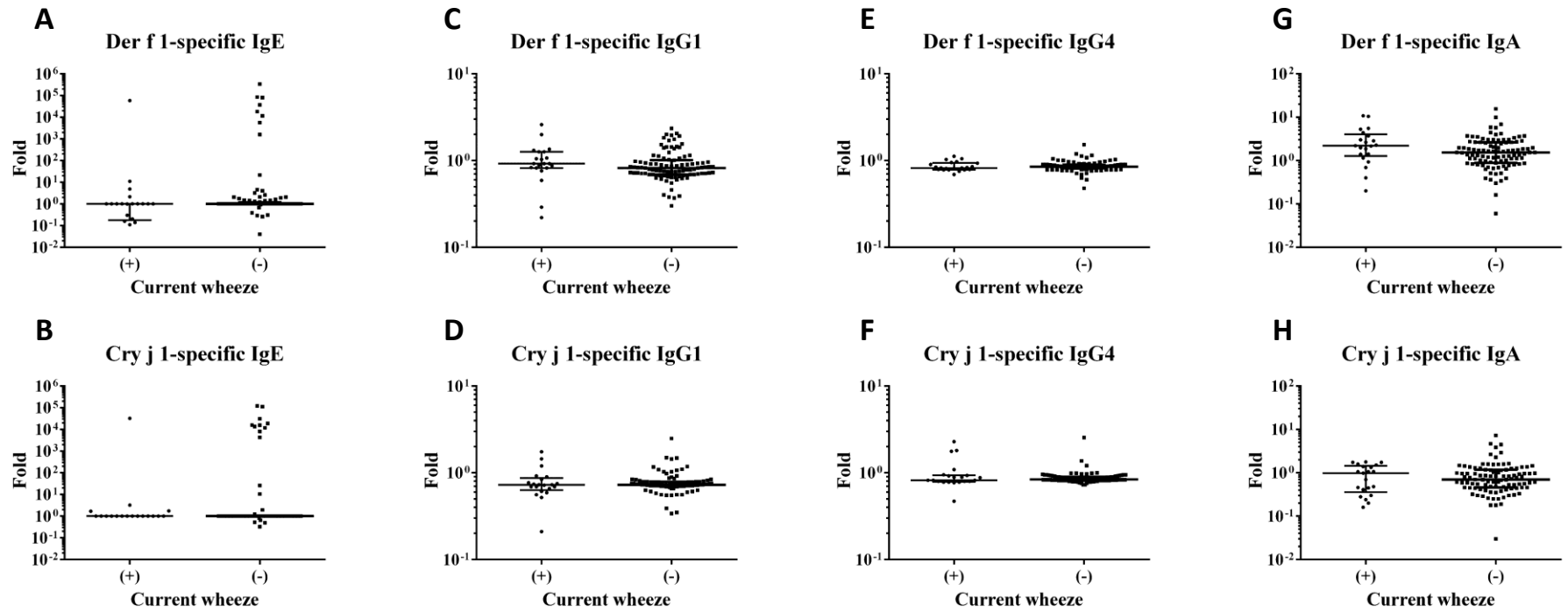
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Supplementary Figure 1.



Supplementary Figure 1. Flow diagram of participants in this study.

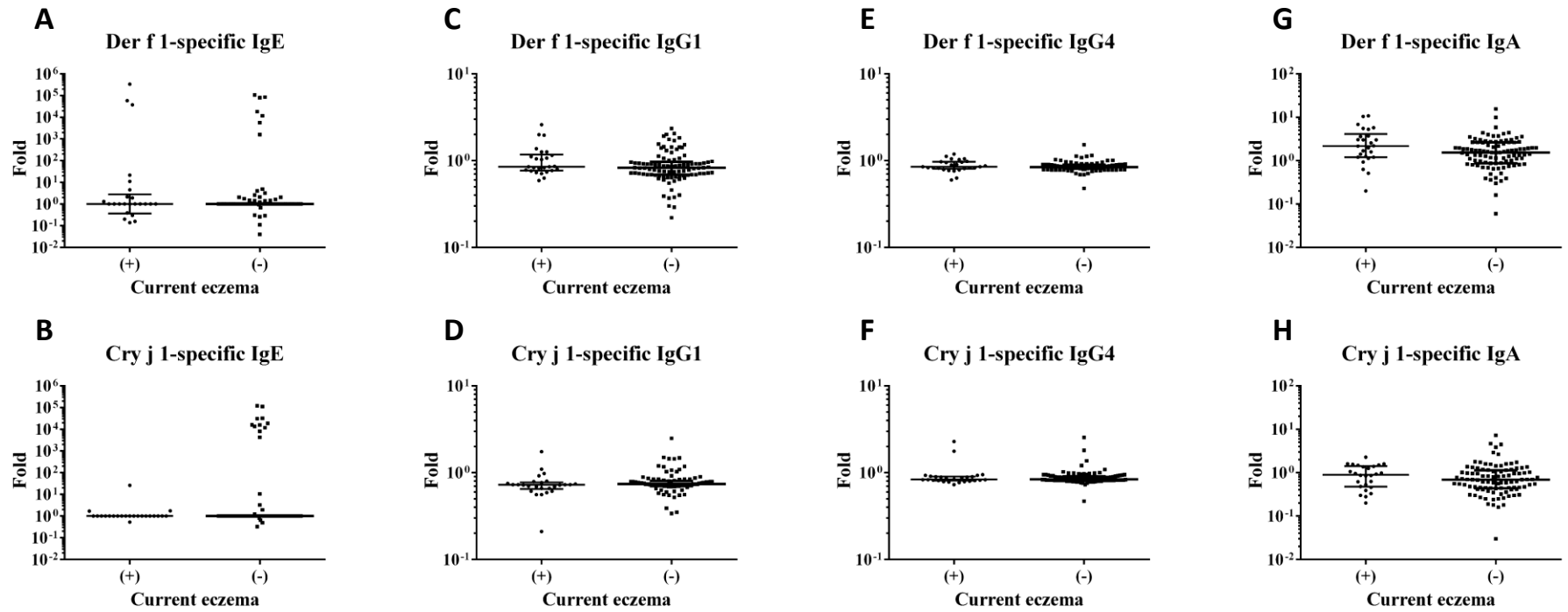
Supplementary Figure 2.



Supplementary Figure 2. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to 8 years and the presence of current wheeze at the age of 6 years.

Comparison between children with current wheeze (+) and children without wheeze (-) at the age of 6 years for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from age 6 to 8 years using the Mann–Whitney U test (A to H). The long bar at the center of the line that runs through the center of each dot represents the median and short bars above and below it represent the interquartile range. P value < 0.05 represents a significant difference.

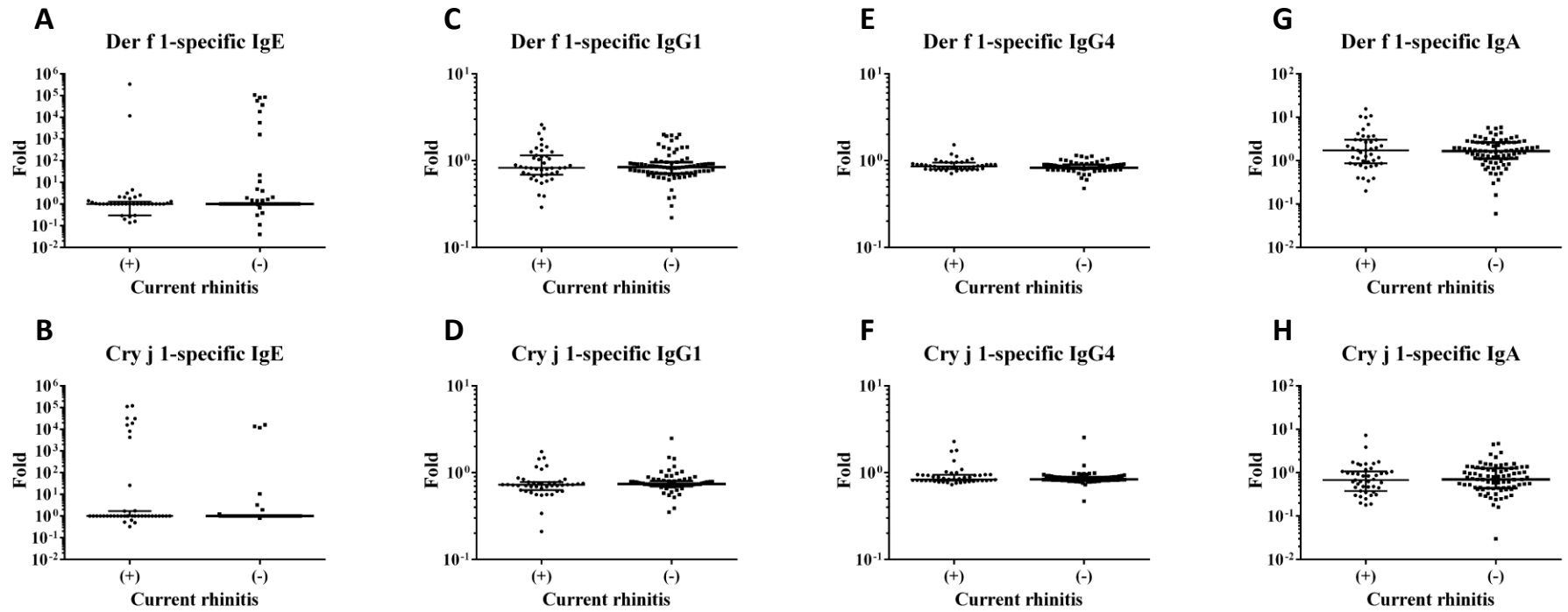
Supplementary Figure 3.



Supplementary Figure 3. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to 8 years and the presence of current eczema at the age of 6 years.

Comparison between children with current eczema (+) and children without eczema (–) at the age of 6 years for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from age 6 to 8 years using the Mann–Whitney U test (A to H). The long bar at the center of the line that runs through the center of each dot represents the median and short bars above and below it represent the interquartile range. P value < 0.05 represents a significant difference.

Supplementary Figure 4.



Supplementary Figure 4. Association between the fluctuation of Der f 1- and Cry j 1-specific immunoglobulins from age 6 to 8 years and the presence of current rhinitis at the age of 6 years.

Comparison between children with current rhinitis (+) and children without rhinitis (-) at the age of 6 years for each fold of Der f 1- and Cry j 1-specific immunoglobulins (IgE, IgG1, IgG4, and IgA) from age 6 to 8 years using the Mann–Whitney U test (A to H). The long bar at the center of the line that runs through the center of each dot represents the median and short bars above and below it represent the interquartile range. P value < 0.05 represents a significant difference.