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Ⅴ．研究成果の刊行物・別刷

（主なもの）

All patients using off-label medication must be informed about the legal consequences, read the manufacturer's consumer information, discuss it with their doctor and give written informed consent.

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Key words: *hyperhidrosis axillaris, quetiapine, social phobia*

Conflicts of interest: none declared.

Proactive treatment appears to decrease serum immunoglobulin-E levels in patients with severe atopic dermatitis

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MADAM, Atopic dermatitis (AD) is a chronic inflammatory skin disease with a broad spectrum of clinical manifestations.[1] Central to the pathogenesis of AD are immunological dysregulation of T-cell function and, frequently, elevated immunoglobulin (Ig) E levels.[2] In severe AD, total serum IgE level can be increased up to 10 000 IU mL^{-1} or even more and may cause several problems in treating allergy.

Recently, proactive treatment, which is long-term, low-dose intermittent application of anti-inflammatory agents to the previously affected skin together with daily application of emollients to unaffected areas, was reported to have several clinical advantages.[3–7] However, there have been no published reports regarding the relationship between IgE level and a proactive treatment approach to AD.

To investigate whether proactive treatment changes serum IgE level or not, we conducted a retrospective study of patients with moderate to severe AD who were treated and followed up in the Division of Allergy, National Center for Child Health and Development (Tokyo, Japan). The inclusion criteria were: (i) admission between January 2004 and July 2007; and (ii) an IgE level above 100 IU mL^{-1} at the first visit, with re-evaluation at least once more after 1–2 years.

In the remission–induction phase, patients applied topical corticosteroids twice daily every day to all affected body areas until visible inflammation disappeared. The maintenance phase consisted of proactive intervention using topical corticosteroids after twice daily skin washing and continuous environmental control: 0·12% betamethasone valerate was applied to the body and extremities, 0·1% hydrocortisone butyrate on the face. According to the tapering protocol of our division (Fig. S1; see Supporting information), the patients were advised to use topical corticosteroids intermittently and proactively on all previously identified affected areas. None of the patients received any additional systemic therapy.

If the patient maintained proactive therapy for 2 years, he or she was assigned to the 'proactive treatment group'. If the patient discontinued the recommended proactive treatments and treated symptoms reactively, he or she was assigned to the 'reactive treatment group'.

Scoring Atopic Dermatitis (SCORAD),[8] safety assessments and height measurements were performed at each hospital visit. Serum total IgE and food-specific IgE levels using an ImmunoCAP Specific IgE kit (Phadia AB, Uppsala, Sweden) were evaluated.

Forty-five patients satisfied the inclusion criteria. All patients achieved remission within 1–2 months of in-hospital treatment. Twenty-five patients continued twice-a-day skin care and proactive treatment and were assigned to the proactive treatment group. Twenty patients failed to continue proactive treatment and were assigned to the reactive treatment group (Fig. S2; see Supporting information). The mean age ± SD was 4·3 ± 3·3 years (proactive group) vs. 5·0 ± 4·0 (reactive group). Baseline SCORAD median (interquartile range) was 82·2 (68·2–85·6) vs. 79·5 (66·8–91·4). Baseline total IgE was 2442 (1263–8330) IU mL^{-1} vs. 2081 (1059–6980). All parameters measured did not show significant differences (Table S1; see Supporting information), but the mean worst SCORAD in the proactive group was lower than that in the reactive group. The application frequency of topical steroid necessary to maintain a clear status was titrated in the proactive group patients, usually 1 day in a week (48% of the proactive group) or 2 days in a week (also 48%).

Serum IgE titre was significantly decreased in the proactive treatment group compared with the reactive treatment group ($P < 0·01$, Mann–Whitney U-test, Fig. 1). In addition, the food-specific IgE level was significantly decreased in the pro-

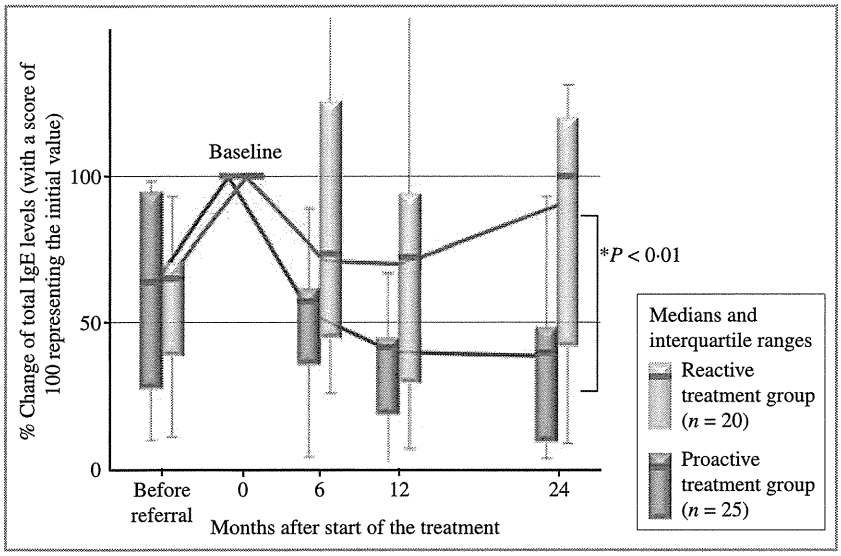

Fig 1. Percentage changes of total immunoglobulin (Ig) E levels during the treatment. Baseline (just before the start of inpatient treatment) total IgE of each patient was determined as 100%. In the proactive group, the total IgE level was reduced by our comprehensive treatment and IgE levels at 2 years after treatment commencement were significantly lower than in the reactive group. *Significant differences between the two groups after 2 years ($P < 0{\cdot}01$, Mann–Whitney U-test).

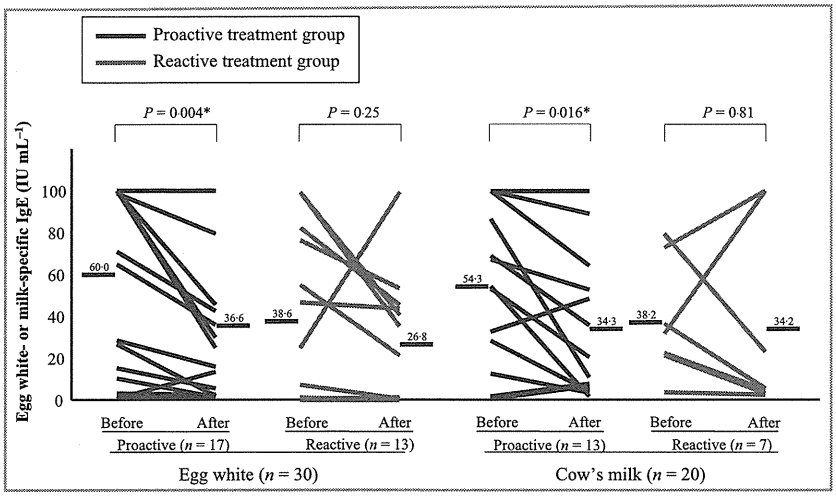

Fig 2. Changes in egg white- and milk-specific immunoglobulin (Ig) E levels after the start of treatment. Food-specific IgE levels in the patients in the proactive treatment group (blue line) decreased significantly during follow-up. No significant decreases were seen in the reactive treatment group patients (red lines). *Significant difference in Wilcoxon signed rank test.

active group compared with the reactive group (Wilcoxon signed rank test, Fig. 2).

There were no serious adverse events in any of the patients during treatment. Eight patients (32%) in the proactive treatment group experienced fungal infection (two patients in reactive group). Skin thinning and striae formation were not seen in any patients. Hypertrichosis was suspected in some patients before titration of frequency of topical steroid, but normalized when the frequency of topical steroid was decreased to twice a week or less. The mean height velocity SD score was relatively high in both groups and did not show any difference.

Inflamed skin of AD bears several kinds of inflammatory cells, including T-helper 2 cells which produce interleukin (IL)-4 and IL-13 and lead to production of IgE in cooperation with B cells. IgE might be synthesized in inflamed skin, its regional lymph node and elsewhere in the body. One can imagine that suppression of inflammatory cells in the skin could lead to disruption of the IgE synthesis mechanism.

We used proactive therapy for the management of severe AD and the serum IgE level appeared to decrease. Continuous close adherence to the proactive treatment may suppress inflammatory cells and lower the serum IgE level. Decrease of egg white- and milk-specific IgE levels was also seen, and may play an important role in alleviating food allergies. This is the first report to evaluate the effectiveness of proactive therapy in reducing the serum IgE level.

Because this study was retrospective and was designed using therapy adherence as a discriminating factor for assigning the patients, we hope to execute a prospective randomized controlled trial. We also want to understand the mechanism to decrease serum IgE and to change the clinical course of food allergy.

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Key words: *atopic dermatitis, food allergy, immunoglobulin E, proactive therapy, topical corticosteroid*

Conflicts of interest: none declared.

Supporting information

Additional supporting information may be found in the online version of the article:

Fig S1. Our standard protocol for proactive treatment of atopic dermatitis.

Fig S2. Patient flow.

Table S1. Stratified analysis of the two groups.

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An unusual case of granulomatous cutaneous T-cell lymphoma showing subcutaneous/muscular involvement and a 5q33.1 deletion

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MADAM, Granulomatous slack skin (GSS) and granulomatous mycosis fungoides (GMF) are classified as subtypes of mycosis fungoides (MF) according to the recent classifications of haematolymphoid tumours.[1,2] GSS clinically manifests as bulky, infiltrated folds of lax skin in flexural areas, histologically characterized by a granulomatous T-cell infiltrate with abundant macrophages, multinucleated giant cells and loss of elastic fibres.[3] We describe an unusual case of granulomatous cutaneous T-cell lymphoma (CTCL) clinically presenting without typical MF cutaneous lesions and showing histopathological features consistent with GSS, with the absence of elastolysis. Array-based comparative genomic hybridization (aCGH) was also performed showing a unique loss of 5q33.1.

A 34-year-old white man presented with a 5-year history of isolated, deep and painful nodules, rapidly enlarged and localized on the right part of the trunk (Fig. 1a), on the right buttock, on the left thigh (Fig. 1b), left knee and left shoulder. A complete physical examination did not reveal any MF-like typical aspects or lymph node enlargement. His medical and family history was unremarkable. Two previous cutaneous biopsies were not diagnostic for CTCL.

When he presented to us, another biopsy was taken from a skin lesion, and previous histological specimens were reviewed. They showed a focal granulomatous lymphoid infiltrate localized in the dermis and hypodermis (Fig. 1c) and a dense lymphoid infiltrate containing multinucleated giant cells (Fig. 1d) forming granulomas in the subcutis and fascia (Fig. 1e). The atypical proliferating lymphocytes showed small to medium-sized cerebriform and hyperchromic nuclei, typically surrounding the multinucleate giant cells and forming rosette-like features (Fig. 1f); there was also evidence of mild lymphophagocytosis. Immunohistochemically, tumour cells were CD2++, CD3+ (Fig. 2a), CD4+, CD5+/−, CD7−, CD8−, CD30−, CD45RO+, interleukin (IL)-17− and Ki-67+ (Fig. 2b). Multinucleate giant cells were positive for IL-17 (Fig. 2c), interferon (IFN)-γ, tumour necrosis factor (TNF)-α, CD68 and CD163 markers.

Molecular analysis revealed a monoclonal T-cell receptor-γ gene rearrangement (primer V2a, V1-8). A histological diagnosis of GSS was made. Complete haematological staging revealed no abnormalities. Magnetic resonance imaging and a thoracoabdominal computerized tomography scan of the cutaneous lesions showed involvement of only subcutaneous tissue and the muscular layer by the infiltrate.

Genotypic studies of the skin biopsy by aCGH showed a loss of only 5q33.1 containing the *IL17B* and *PCYOX1L* genes

TABLE I. Sensitivity and specificity* for Ara h 2 and whole peanut extract

Test	Cutoff point (kU_A/L)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
Ara h 2	0.30	100.00	90.20	93.75
	0.32	100.00	94.12	95.00
	0.35	100.00	96.08	97.50
	0.38	96.55	96.08	96.25
	0.40	93.10	98.04	96.25
	0.55	93.10	100.00	97.50
	0.87	89.66	100.00	96.25
Whole extract	0.35	96.55	26.92	51.85
	3.91	79.31	84.62	82.72
	5.00	75.86	90.38	85.19
	5.30	75.86	94.23	87.65
	5.96	72.41	94.23	86.42
	7.81	72.41	96.15	87.65
	15.00	55.17	96.15	81.48
	43.86	34.85	98.08	75.31

Analysis included all children with available data (81 for sIgE to whole peanut extract and 80 for sIgE to Ara h 2).
*Sensitivity refers to the proportion of subjects who have peanut allergy and give positive test results. Specificity refers to the proportion of subjects without the target condition and a negative test result for peanut allergy.

peanut allergy and 50 are peanut-tolerant. By using sIgE to component Ara h 2 with a cutoff point of 0.35 kU_A/L, all children with peanut allergy would be correctly classified. The specificity of this test is given as 96.1% (Table I). In this example we expect 2 children who are not allergic to peanuts to be misclassified as having peanut allergy and the other 48 children to have a negative result. By using this cutoff point, 97.5% of the population is correctly classified. A similar proportion of children would be correctly classified by using a cutoff point of 0.55 kU_A/L; however, in this case 3 children with peanut allergy would be misclassified as tolerant. This cutoff point corresponds to a gain in specificity (100%) but a loss in sensitivity (93.1%). Given the importance of not misdiagnosing children with peanut allergy as being tolerant, we propose that the optimal cutoff point in our population is 0.35 kU_A/L.

The cutoff for whole peanut sIgE of 5.30 kU_A/L provides the maximum proportion of correctly classified subjects (87.6%), with a sensitivity of 75.9% and a specificity of 94.2%. However, approximately 24% of children with peanut allergy would be inappropriately classified as peanut-tolerant. The cutoff of 15 kU_A/L has excellent specificity, with 96.2% of children at greater than this level being correctly classified as allergic; however, this decision point has relatively poor sensitivity, with almost half of the subjects with peanut allergy being classified as tolerant. These data suggest that in our population the quantification of whole peanut sIgE has lower accuracy in discriminating peanut allergy from tolerance compared with quantification of sIgE to Ara h 2.

In conclusion, having identified sIgE to Ara h 2 as an important predictor of clinical reactivity to peanut using microarray technology,[5] we have now demonstrated the value of its quantification using a routinely available laboratory test. Among school-aged children in the United Kingdom, a cutoff of 0.35 kU_A/L Ara h 2 IgE confers 100% sensitivity and 96.1% specificity. By using this cutoff point, 97.5% of the subjects in our study population were correctly classified, with all children with peanut allergy given the correct classification. The importance of Ara h 2 has been suggested in studies from other Central and Northern European countries[7,8]; however, in other populations and geographic areas, IgE to other components might be relevant (eg, Ara h 9 in the Mediterranean[9]). Our findings need to be replicated in other populations and age groups before general application.

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Four distinct subtypes of non–IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms

To the Editor:

Although most food allergies are IgE-mediated, there are a number of non–IgE-mediated gastrointestinal food allergies that affect mainly infants and young children.[1,2] Because most such

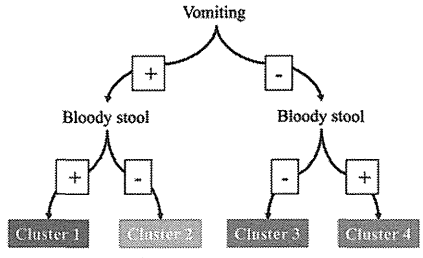

FIG 1. Tree analysis using 2 variables (vomiting and bloody stool at initial presentation) enables assignment of patients into 4 clusters.

patients develop the allergy more than 2 hours after ingestion of the offending food and show negative skin prick tests and/or absence of serum specific IgE against the offending food, these allergies are thought to be cell-mediated. However, the precise pathogenetic mechanisms of these disorders remain poorly understood. Several investigators have described different subtypes of non–IgE-mediated gastrointestinal food allergies: food protein-induced enterocolitis syndrome (FPIES),[3] food protein-induced proctocolitis syndrome (hereafter referred to as "proctocolitis"),[4] food protein-induced enteropathy syndrome (hereafter referred to as "enteropathy"),[5] celiac disease, and allergic eosinophilic gastroenteropathies.

Presumably because the main target organ of these syndromes is the gastrointestinal tract, patients with non–IgE-mediated gastrointestinal food allergies often exhibit similar symptoms, such as vomiting and diarrhea. However, it remains unclear whether these syndromes have the same pathogenesis and merely differ in severity, or whether the pathogenesis of each is distinct, meaning that they should be classified as separate clinical entities.

We applied cluster analysis to the clinical and laboratory findings to characterize these non–IgE-mediated food allergies and determine whether they are made up of distinct clinical entities. A total of 176 patients with detailed clinical records who had been registered in the database of the Japanese Research Group for Neonatal, Infantile Allergic Disorders from 2007 to 2010 were enrolled. Among them, 136 patients fulfilled 3 of the Powell[6] criteria: (1) a switch to therapeutic milk led to resolution of symptoms, (2) differential diagnosis from other disorders was possible, and (3) there was verified body weight gain. Definitive diagnosis was possible for 46 patients by oral food challenge tests that were performed after complete resolution of the initial symptoms (see this article's Fig E1 in the Online Repository at www.jacionline.org). These 46 patients were subjected to further analysis. Details of food challenge test are available in this article's Food challenge test, method section in the Online Repository at www.jacionline.org. Our total cohort included 15 patients who developed the most severe reactions, including ileus, shock, and developmental retardation. The clinical characteristics of those patients are summarized in this article's Table E1 in the Online Repository at www.jacionline.org. Because of the medical and ethical justification, even though these patients fulfilled 3 elements of the Powell[6] criteria, oral challenge tests were not performed. Thus, these patients were excluded from this cluster analysis of 46 patients. This study was approved by the Ethics Committee of the National Center for Child Health and Development.

We omitted clinical and laboratory findings found only in a few patients and finally selected 5 variables: birth weight, age at first presentation (days after birth), severity of vomiting (ranked as 0, none; 1, 1-2 times a day; 2, 3-5 times a day; and 3, more than 5 times a day or bilious vomiting) and severity of bloody stool (0, none; 1, spotty; 2, intermediate; and 3, massive) at first presentation, and milk-specific IgE antibody titer (class 0-6). Unsupervised cluster analysis and discriminant analysis were performed by using SPSS version 18 software (SPSS, Inc, Chicago, Ill). The Wald minimum-variance hierarchic clustering method was performed by using an agglomerative (bottom-up) approach and Ward's linkage. The squared Euclidean distance was used as a proximity measure. Values were transformed by a maximum magnitude of 1. ANOVA, the Tukey-Kramer test, and the χ^2 test were used for parametric continuous, nonparametric continuous, and categoric variables. As a result, the 46 definitively diagnosed patients were classified into 4 distinct clusters, and a dendrogram was generated (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Stepwise discriminate analysis identified the 2 strongest discriminatory variables for cluster assignment: vomiting and bloody stool (Fig 1). Cluster 1 was the patient group with vomiting and bloody stool at initial presentation. Cluster 2 had vomiting but not bloody stool. Cluster 3 had neither vomiting nor bloody stool. Cluster 4 had bloody stool but not vomiting. One patient initially assigned to cluster 3 in fact had clear bloody stool, and was thus reassigned to cluster 4 in accordance with Fig 1. As a result, clusters 1 through 4 consisted of 14, 16, 5, and 11 patients, respectively.

Table I presents the demographic data for each cluster. Cluster 3 showed a significantly lower birth weight and later onset of disease. Clusters 1 and 4 both had bloody stool, but they had normal birth weight and a somewhat earlier onset (median of 7 days after birth).

The laboratory data generated within the initial several days after onset showed that the peripheral blood eosinophil ratio was high in all clusters, with no significant differences among them. In contrast, eosinophils were found in the stool mainly of patients in clusters 1 and 4, in which all patients, by definition (Fig 1), had bloody stool. The presence of eosinophilia suggests that patients with non–IgE-mediated gastrointestinal food allergies tend to have a T_H2-prone immune deviation at baseline, but some additional factors such as overproduction of eosinophil-attracting chemokines are probably necessary to induce immune responses involving eosinophils in the gut (see this article's Fig E3 in the Online Repository at www.jacionline.org).

A positive milk-specific IgE antibody titer was observed in 37% of the patients, with no statistically significant differences among any of the clusters. In addition, almost all symptoms at initial presentation as well as in oral food challenge tests began to manifest at more than 2 hours after ingestion of the offending food, whereas no patients developed typical IgE-mediated symptoms such as urticaria or wheeze. These results strongly suggest that the presence of milk-specific IgE antibody neither causes the gastrointestinal symptoms nor rules out a diagnosis of non–IgE-mediated gastrointestinal food allergy.

One of the most notable findings of this study was the remarkably high reproducibility of symptoms provoked in the oral food challenge tests and those found at the initial presentation in all 4 clusters, even though the oral challenge tests were performed several months after the initial presentation (Table I). This observation suggests that the upper or lower gastrointestinal tract–specific hypersensitivity and perhaps the responsible

TABLE I. Demographic data of the patients (total = 46) whose diagnosis was confirmed by oral food challenge tests

Clinical characteristics	Cluster 1 (n = 14)		Cluster 2 (n = 16)		Cluster 3 (n= 5)		Cluster 4 (n = 11)		*P* value
Birth weight (g)		2642 (2410-3030)		2745 (2223-3079)		1008 (907-2491)		2678 (2512-3170)	.03*
Male/female (n)		6/8		9/7		2/3		5/6	.95
Initial presentation									
Day of onset		7.5 (3-23)		16.5 (9.5-33.5)		37 (8.5-132)		7 (2-56)	.17
Vomiting (%)		100		100		0		0	.000*
Bloody stool (%)		100		0		0		100	.000*
Fever (%)		7.1		18.8		20.0		0	.45
(Laboratory data)†	n		n		n		n		
Blood eosinophil ratio (%)‡	13	15 (3.0-23)	14	7 (3.9-19.3)	5	27 (3.2-39.3)	11	14 (4.5-25)	.63
WBC ($\times 10^3$/mL)§	13	18.4 (13.7-22.7)	14	15.7 (11.4-21.9)	5	21.8 (11.0-27.7)	11	13.1 (8.2-18.3)	.64
Total IgE (IU/mL)‖	14	5.2 (4.8-28.3)	16	11.4 (5.0-80.8)	5	7.4 (5.5-653.5)	10	5.0 (2.0-5.8)	.36
Positive for milk-specific IgE (class ≥1) (%)	14	57	16	37.5	5	40	11	9	.28
C-reactive protein (% positive, ≥0.5)	13	46	14	50	5	40	10	30	.47
Stool eosinophil (% positive)	8	50	6	33	3	0	7	100	.01*
Diet (reaction to each milk, %)									
Cow's milk	14	100	16	100	5	100	10	100	1.00
Breast milk	8	38	7	0	2	50	7	27	.40
Hydrolyzed formula	9	0	10	20	2	0	8	63	.02*
Oral food challenge test									
Onset of reaction (h)		6 (1.8-12)		10 (2-24)		48 (24-60)		24 (24-48)	.17
Vomiting (%)		85.7		81.3		0		9.1	.000*
Bloody stool (%)		28.6		6.3		0		72.7	.001*
Diarrhea (%)		21.4		31.3		60.0		18.2	.33

WBC, White blood cell count.
Data are shown as the median and the interquartile range.
*$P < .05$.
†*n,* Number with medical records.
‡Normal range of blood eosinophils is 0% to 4%. However, it is known to rise to some degree in the neonatal period, especially in low-birth-weight infants.[10]
§Normal range of WBC in neonatal period is 7.0 to 25.0 $\times$ $10^3/\mu L$.
‖Normal range of total IgE in infantile period is less than 20 IU/mL.

immune cells remain in the same part of the gastrointestinal tract even after several months' remission.

Because the patients in clusters 1 and 2 had vomiting that was provoked at relatively early time points, they are likely to be diagnosed as having FPIES, although the bloody stool and eosinophilia seen mainly in cluster 1 patients were not emphasized in earlier reports.[7,8] The nearly simultaneous manifestation of vomiting and bloody stool suggests that FPIES may affect both the upper and lower gastrointestinal tracts.

The main symptoms of the patients in cluster 3 were poor weight gain and diarrhea and were similar to those found in patients with enteropathy. The significantly lower birth weight and marked eosinophilia characteristically found in cluster 3 patients imply the involvement of immature gastrointestinal function in the pathogenesis of this syndrome.

Bloody stool was the main symptom of the patients in cluster 4. Some patients in this cluster had no systemic manifestation other than bloody stool, whereas others also had diarrhea and/or poor weight gain. Therefore, these patients may be diagnosed as having proctocolitis or early onset of allergic eosinophilic gastroenteropathies, respectively. However, the pathogenetic similarity and/or disparity of proctocolitis and allergic eosinophilic gastroenteropathies need to be studied further.

In our cohort, 3 children with exclusive breast-feeding have developed FPIES. This information is available in this article's Breast-feeding and FPIED section in the Online Repository at www.jacionline.org.

Elevated serum C-reactive protein levels were found in 30% to 50% of patients with non–IgE-mediated gastrointestinal food allergies. In addition, some patients developed a fever during oral food challenge tests, suggesting that TNF-α and other proinflammatory cytokines may be involved in the pathogenesis of these syndromes.[9]

To confirm the results of cluster analysis, we performed the same analysis for the aforementioned 136 patients who fulfilled 3 of the Powell[6] criteria (consisting of the 46 patients definitively diagnosed by oral food challenge and 90 patients not subjected to oral food challenge; Fig E1). We obtained exactly the same results: the patients were assigned to 4 clusters in accordance with the tree analysis shown in Fig 1. The patients' demographics (see this article's Table E2 in the Online Repository at www.jacionline.org), birth weight (see this article's Fig E4 in the Online Repository at www.jacionline.org) and peripheral blood eosinophils (see this article's Fig E5 in the Online Repository at www.jacionline.org) confirmed the earlier cluster analysis findings.

In our ongoing cohort, 52% of the patients acquired tolerance to the offending food by 1 year of age, 88% by 2 years, and 94% by 3 years. Therefore, assuming that identification and elimination of the offending food had been done properly, it can be assumed that most patients outgrew their allergy by the age of 2 to 3 years. On the other hand, just like patients with severe IgE-mediated food allergy, patients with non–IgE-mediated gastrointestinal food allergies may develop severe reactions

(Table E1). Thus, early diagnosis is very important, and refinement of the diagnostic method is truly necessary.

Our findings clearly demonstrated that patients with these non–IgE-mediated gastrointestinal food allergies showed similar T_h2-prone laboratory data (eosinophilia and presence of specific IgE antibody), but the disease entities of each cluster had distinct clinical features and may have different pathogenetic mechanisms.

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FOOD CHALLENGE TEST, METHOD

Generally, oral challenge tests were performed at 4 to 6 months of age. First, 4 mL milk/kg body weight was administered. If no reaction occurred, the dose of milk was increased daily until symptoms manifested. If the reaction had been evoked by a very small volume of milk in the initial presentation, the test was started using a lesser volume to avoid a serious reaction. Because of the medical and ethical justification for oral food challenge tests, patients with the most severe reactions were excluded from the initial cluster analysis. Their clinical characteristics are summarized in Table E1.

BREAST-FEEDING AND FPIES

Six of the 46 patients were exclusively breast-fed. Three of them were included in cluster 1 and can be diagnosed as FPIES. Those 3 patients showed a positive reaction to cow's milk as well as breast milk even after their mothers stopped consuming milk products. These patients also developed symptoms when orally challenged with rice and/or soy. Therefore, these findings indicate that not only proctocolitis but also FPIES can develop even in children who are exclusively breast-fed. A recent case report supports our findings.[E1]

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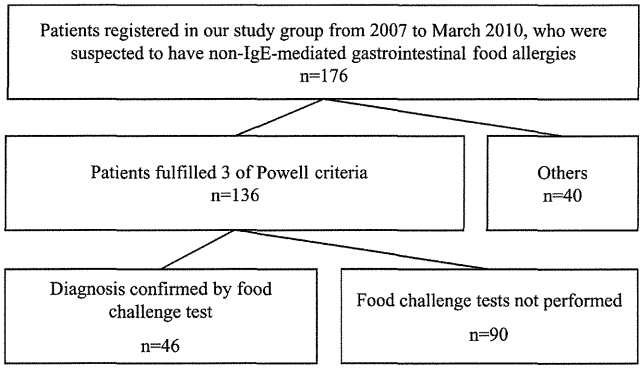

FIG E1. A total of 176 patients with gastrointestinal symptoms who were suspected of having non–IgE-mediated allergy from 1999 to 2009 were registered by doctors of the Japanese Research Group for Neonatal, Infantile Allergic Disorders. Of them, 136 patients fulfilled elements 1 through 3 of the Powell criteria. Forty-six patients underwent food challenge tests and had a positive result, whereas the remaining 90 patients were not tested. Seventeen patients showed no reaction in the oral challenge tests. However, it was unclear whether this was because the patients had outgrown their allergy or because of misdiagnosis. Those 17 patients were excluded from further analysis in this study.

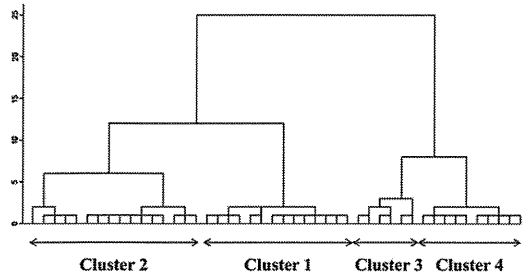

FIG E2. The 46 patients definitively diagnosed with non–IgE-mediated food allergies were analyzed for 5 variables by using an agglomerative (bottom-up) approach and Ward's linkage, and a dendrogram was generated.

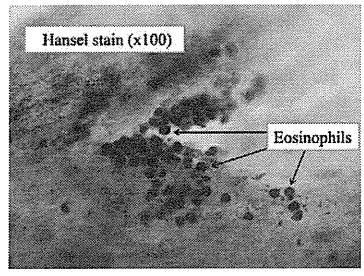

FIG E3. Detection of accumulations of eosinophils in the stool mucus. The mucous part of the stool was thinly smeared on a glass slide and stained by using Hansel stain. The stool sample was taken from a patient in cluster 2 after a positive food challenge test. Representative images were found in a total of 13 patients (Table I).

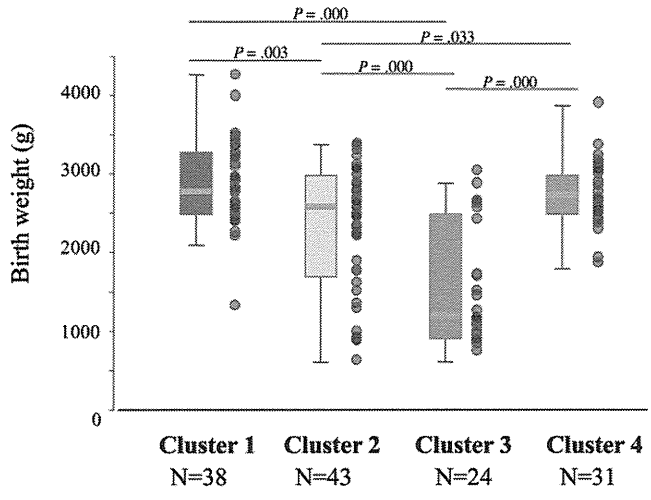

FIG E4. The birth weights in each cluster of the 136 patients who fulfilled 3 elements of the Powell criteria for a non–IgE-mediated allergy are shown.[E2] The birth weights in cluster 3 were significantly lower than in the other clusters. Moreover, 2 subgroups seem to be identified in cluster 3: a lower birth weight group and a normal birth weight group.

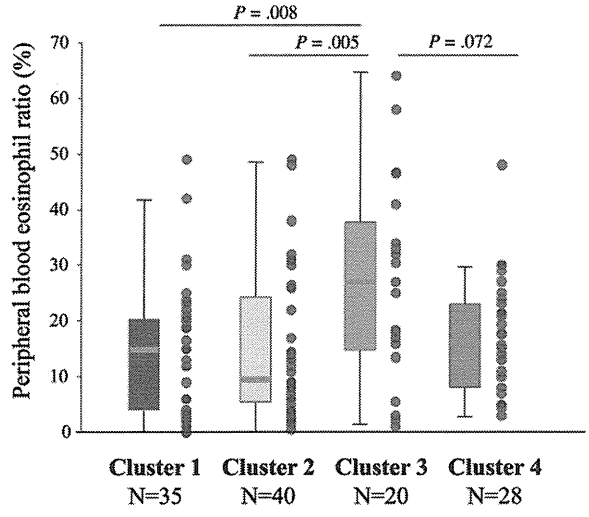

FIG E5. The peripheral blood eosinophil ratios in each cluster of the 136 patients who fulfilled 3 elements of the Powell criteria are shown.[E2] Although eosinophilia was found in all 4 clusters, the eosinophil ratios of the patients in cluster 3 were significantly higher than those of the patients in clusters 1 and 2.

TABLE E1. Clinical features of most severe cases of non–IgE-mediated gastrointestinal food allergies*

Patient no.	Sex	Cluster	Complication	Day of onset	Diet right before the onset of complications	Remarks
1	F	1	Ileus	8	Cow's milk 7 d	
2	M	1	Ileus	5	Cow's milk 3 d, breast milk 6 d	Relieved by surgical operation
3	F	1	Ileus	8	Breast milk 9 d	Relieved by surgical operation
4	F	1	Shock	2	Cow's milk 2-3 times	Massive bloody stool, blood infusion required
5	F	1	Shock	21	Breast milk 18 d	Massive bloody stool, disseminated intravascular coagulation
6	F	2	Ileus	14	Breast milk 2 d	
7	F	2	Shock	36	Breast milk 30 d	Apnea, vomiting
8	M	2	Shock	30	Cow's milk 50 mL by chance	Vomiting
9	M	2	Shock	241	Soy food 2-3 times	Vomiting and diarrhea, ICU admission
10	M	3	Ileus	61	Breast milk 45 d	Cholestasis
11	F	3	Shock	22	Cow's milk 21 d, breast milk 21 d	ICU admission
12	F	3	Severe weight loss	12	Breast milk several months	Developmental retardation
13	M	3	Severe weight loss	46	Cow's milk 30 d, breast milk 30 d	Developmental retardation
14	F	4	Ileus	2	Cow's milk 6 d, breast milk 3 d	Stenosis of sigmoid colon
15	F	4	Ileus	7	Cow's milk 10 d	

F, Female; *ICU,* intensive care unit; *M,* male.
*These patients fulfilled 3 elements of the Powell criteria,[E2] but oral challenge tests were not performed.

TABLE E2. Demographics of the 136 patients who fulfilled 3 elements of the Powell criteria[E2]

Clinical characteristics		Cluster 1 (n = 38)		Cluster 2 (n = 43)		Cluster 3 (n = 24)		Cluster 4 (n = 31)	*P* value
Birth weight (g)		2823 (2501-3267)		2581 (1779-3016)		1363 (1023-2611)		2778 (2512-3100)	.000 *
Male/female		19/19		28/15		13/11		12/19	.16
Initial presentation									
Day of onset		6 (4-8)		29 (7.5-52)		16.5 (9.5-37.5)		7 (2-35)	.01*
Vomiting (%)		100		100		0		0	.000*
Bloody stool (%)		100		0		0		100	.000*
(Laboratory data)†	**n**		**n**		**n**		**n**		
Blood eosinophil ratio (%)	35	15 (3.5-21.0)	40	9 (5.3-25.0)	20	26 (14.1-39.3)	28	17 (8.5-23.8)	.005*
WBC ($\times 10^3$/mL)	32	18.7 (14.5-23.5)	40	13.8 (10.4-22.1)	23	15.9 (13.9-24.4)	27	13.9 (11.4-19.5)	.16
Total IgE (IU/mL)	32	5.2 (4.1-23.1)	40	5.8 (4.0-17.8)	22	13.2 (5.5-122.9)	28	5.0 (3.3-6.0)	.001*
Positive for milk-specific IgE (class ≥1) (%)	31	41.9	38	23.7	20	50	27	19	.24
C-reactive protein (% positive, ≥0.5)	36	61	40	45	20	70	27	33	.69

WBC, White blood cell count.
Data are shown as the median and the interquartile range.
*$P < .05$.
†*n*, Number with medical records.

Genome-Wide Association Study Identifies *HLA-DP* as a Susceptibility Gene for Pediatric Asthma in Asian Populations

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Abstract

Asthma is a complex phenotype influenced by genetic and environmental factors. We conducted a genome-wide association study (GWAS) with 938 Japanese pediatric asthma patients and 2,376 controls. Single-nucleotide polymorphisms (SNPs) showing strong associations ($P<1\times10^{-8}$) in GWAS were further genotyped in an independent Japanese samples (818 cases and 1,032 controls) and in Korean samples (835 cases and 421 controls). SNP rs987870, located between *HLA-DPA1* and *HLA-DPB1*, was consistently associated with pediatric asthma in 3 independent populations ($P_{combined}=2.3\times10^{-10}$, odds ratio [OR] = 1.40). *HLA-DP* allele analysis showed that *DPA1*0201* and *DPB1*0901*, which were in strong linkage disequilibrium, were strongly associated with pediatric asthma (*DPA1*0201*: $P=5.5\times10^{-10}$, OR = 1.52, and *DPB1**0901: $P=2.0\times10^{-7}$, OR = 1.49). Our findings show that genetic variants in the *HLA-DP* locus are associated with the risk of pediatric asthma in Asian populations.

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Introduction

Asthma is the most common chronic disorder in children, and asthma exacerbation is an important cause of childhood morbidity and hospitalization. The prevalence of childhood asthma in Japan is 5.0% among school children in 2006 [1], and an estimated 300 million people worldwide have asthma [2]. Asthma is characterized by airway hyperresponsiveness and inflammation, tissue remodeling, and airflow obstruction. Infiltration of lymphocytes, mast cells, and eosinophils in the airways cause airway inflammation, and T helper (Th) type 2 cytokines play crucial roles in orchestrating the inflammatory responses; thus, asthma is considered a Th2-type immune disease.

Previously conducted genome-wide association studies (GWAS) for asthma identified association with the loci on chromosomes 17q21 (*ORMDL3* for Caucasian pediatric asthma, odds ratio (OR) = 1.45, $P=1\times10^{-10}$) [3], 5q21 (*PDE4D* for pediatric asthma, OR = 0.6, $P=4.7\times10^{-7}$) [4], 9q21.31 (*TLE4* for Hispanic pediatric asthma, OR = 0.6, $P=6.8\times10^{-7}$) [5], and 1q31 (*DENND1B* for Europeans and African ancestries [6], OR = 0.77 and 1.41, respectively; combined $P=1.7\times10^{-13}$). A GWAS for severe asthma identified association with the region between