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Negative Act d 8 indicates systemic kiwifruit allergy among kiwifruit-sensitized children

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To the Editor:

Kiwifruit allergy appears to be increasing (1). Symptoms of kiwifruit allergy vary from localized oral symptoms to life-threatening anaphylaxis (2, 3).

Kiwifruit allergy is generally diagnosed based on medical history, positive specific immunoglobulin E (IgE) to kiwifruit, and oral food challenge (OFC). It is important to identify biomarkers to differentiate severe symptoms from localized oral symptoms. However, commercially available in vitro tests for specific IgE antibodies to crude kiwifruit extracts cannot predict severe kiwifruit allergy (4, 5). Although the prick by prick test has high sensitivity, it has low specificity and cannot predict severe symptoms (2).

Molecular-based allergy diagnostics is a promising approach to improve the diagnostic accuracy of food allergies. Thirteen kiwifruit allergen components have been registered by the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Subcommittee. However, reports regarding kiwifruit allergen components associated with clinical symptoms are limited.

Act d 1, a cysteine protease, is a major kiwifruit allergen. Sensitization to Act d 1 has been identified as a risk factor for severe kiwifruit allergy (5). Act d 8, a pathogenesis-related protein 10 (PR-10), is a homologue to the major birch pollen allergen Bet v 1. Allergic symptoms elicited by a member of the Bet v 1 family are usually confined to the oral mucosa or angioedema of the lips, which can be explained by heat lability and low resistance to digestion of Bet v 1 homologous

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proteins. Bet v 1 has homology with the major pollen allergen of alder. In Japan, alder grows widely, whereas birch grows in limited locations such as northern island and central mountain area. In these metropolitan areas, some individuals are sensitized to alder and may develop kiwifruit allergy due to cross-reactivity.

While severe symptoms are significantly more likely to occur among young children than in adults (1), the diagnostic utility of specific IgE for detecting pediatric severe kiwifruit allergy has not been investigated (1). Therefore, the aims of this study were to investigate the clinical features of pediatric kiwifruit allergy and to validate the performance of specific IgE response to alder, Bet v 1, and Act d 8 for detecting pediatric severe kiwifruit allergy among children sensitized to kiwifruit.

We retrospectively analyzed, through medical record review, the characteristics, specific IgE response, and clinical symptoms of children with kiwifruit allergy who visited our hospital between 2008 and 2013. Kiwifruit allergy was defined as immediate symptoms, such as urticaria or oral itching, and specific IgE response to kiwifruit ≥ 0.34 kU/L. We measured specific IgE response to alder, Bet v 1, and Act d 8. Specific IgE level was measured by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Sera from all patients were prepared from venous blood samples, and frozen at -20°C until use. Results below the lower limit of quantitation (<0.10 kU/L) were assigned a value of 0.05 kU/L for statistical calculations. We classified kiwifruit allergy into 2 groups based on reaction: local, only mucosal symptoms, such as oral itching or angioedema of the lips (2), and systemic, symptoms such as urticaria, cough, or anaphylaxis (3), according to the study by Fernandez et al. (6). This study was reviewed and approved by the local ethics committee.

Differences in sex, age, associated atopic disorders, and specific IgE levels between the local and systemic groups were assessed by using the Mann–Whitney *U* test, Chi-square test, or Fisher's exact test. The diagnostic value of specific IgE for discriminating between local and systemic reaction was determined by calculating the area under the curve of the receiver operating characteristic (ROC) curve. The Youden index was used to confirm the cutoff value established with the ROC curves. Sensitivity and specificity of specific IgE

for discriminating between local and systemic reaction also were calculated. Logistic regression analysis was performed by using a model with clinical severity as the dependent variable and specific IgE response to alder, Bet v 1, and Act d 8 as independent variables. Each specific IgE response was dichotomized as positive (reference) or negative. Results for systemic reaction were presented as the odds ratio and 95% confidence interval according to each specific IgE response. All models were adjusted for age at blood examination because older age appeared to be associated with pollinosis. Statistical analysis was performed by using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) for Windows, with P values of <0.05 regarded as significant.

A total of 110 children with kiwifruit allergy visited our clinic between 2008 and 2013 (Figure S1). Forty-seven patients were excluded because the interval between age at onset of allergy and age at blood examination was >2 years. Nine additional patients were excluded due to missing data. The remaining 54 patients were included in this study.

The patients' characteristics are summarized in Table S1. We included 33 males and 21 females. Median age at onset was 6 years (range, 1–13 years), while median age at blood examination was 7 years (range, 1–14 years). Median specific IgE value to kiwifruit, alder, Bet v 1, and Act d 8 were 3.5 kU/L, 0.98 kU/L, 0.13 kU/L, and 0.12 kU/L respectively.

In the 54 patients, mucosal symptoms were seen most frequently, appearing in 43 patients (78%); 31 patients (57%) had only mucosal symptoms (Table S2). Skin symptoms were seen in 16 patients (30%), followed by gastrointestinal symptoms in 12 (22%), respiratory symptoms in 5 (9%), and cardiovascular symptoms in 1 (2%).

There were no differences in baseline characteristics between the local and systemic groups in terms of sex, associated atopic disorders, and specific IgE response to kiwifruit (Table 1). Age at onset, age at blood examination, and specific IgE response to alder, Bet v 1, and Act d 8 were significantly lower in the systemic group than those in the local group.

As indicated by the area under the ROC curves, specific IgE response to Act d 8 had the highest accuracy for differentiating between local and systemic reaction, followed by Bet v 1 and alder (Figure 1). Areas under the ROC curves of specific IgE response to Act d 8, Bet v 1, and alder were 0.819, 0.779, and 0.743, respectively. Optimal cutoff points of specific IgE response to Act d 8, Bet v 1, and alder were 0.106 kU/L, 0.138 kU/L, and 1.06 kU/L, respectively. Values below 0.106 kU/L for specific IgE response to Act d 8 could predict systemic reaction with 83.9% sensitivity and 78.3% specificity. Values below 0.138 kU/L for specific IgE response to Bet v 1 could predict systemic reaction with 71.0% sensitivity and 82.6% specificity. Values below 1.06 kU/L for specific IgE response to alder could predict systemic reaction with 67.7% sensitivity and 78.3% specificity.

Uni- and multivariate analyses for specific IgE response associated with systemic reaction are shown in Table 2. Negative specific IgE response to alder, Bet v 1, or Act d 8 was more likely in the systemic group than in the local group, even after adjusting for age at blood examination.

In this study, we evaluated the usefulness of specific IgE response to alder, Bet v 1, and Act d 8 in children with kiwifruit allergy, and validated the performance of IgE for detecting pediatric severe kiwifruit allergy.

Mucosal symptoms were seen in 78% of patients, and 57% had only mucosal symptoms. Mucosal symptoms were seen most frequently, though skin symptoms are usually most common with other food allergies as well as food-dependent exercise-induced anaphylaxis. This finding might be because kiwifruit allergen was inactivated through digestion. In addition, most of the patients were younger children who had other food allergies and could not complain of their mucosal symptoms. On the other hand, in a previous double-blind, placebo-controlled food challenge conducted in adults with kiwifruit allergy, mucosal symptoms were reported in 90% of patients, and 69% had only mucosal symptoms (4). This discrepancy in results may be because their subjects were adults, and there was a large influence of cross-reactivity with pollen (4).

We classified kiwifruit allergy based on local or systemic reaction. Age at onset was significantly lower in the systemic group. However, specific IgE response to kiwifruit was useless for discriminating between local and systemic reaction. Similar results were seen in a previous study conducted in the United Kingdom, regardless of geographic difference (1). In the current study, sensitization to alder was significantly higher in the local group. Allergic rhinitis was common in the local group, but no significant difference was found between groups. This finding could be affected by perennial allergic rhinitis or sensitization to alder without pollinosis.

Sensitization to Act d 1, a major kiwifruit allergen, was identified as a strong risk factor for severe kiwifruit allergy in all ages in a previous study conducted in Europe (5). However, in their study, specific IgE response to Act d 8 was not identified as a risk factor influencing clinical severity of kiwifruit allergy through multivariate analysis. One of the reasons for this discrepancy is that they divided severity into 3 groups, not 2. Other possible reasons include differences in geography and age. In our study, negative specific IgE response to Act d 8 was more likely in the systemic group than in the local group. In other words, negative specific IgE response to Act d 8 was a risk factor for severe kiwifruit allergy. Furthermore, negative specific IgE response to alder or Bet v 1 was also a risk factor for severe kiwifruit allergy. To the best of our knowledge, this is the first study to identify negative specific IgE response to alder, Bet v 1, or Act d 8 as a risk factor for pediatric severe kiwifruit allergy among children sensitized to kiwifruit even after adjusting for age at blood examination.

Mastorilli pointed out that kiwifruit allergy is not specifically related to any panallergen (7). Our study did not prove that sensitization of PR-10 leads to development of kiwifruit allergy, but we did show that severity of kiwifruit allergy is related to sensitization of PR-10: most children with kiwifruit allergy without sensitization of PR-10 will present severe allergic symptoms. On the other hand, Act d 12 and Act d 13, kiwifruit seed storage proteins, have been identified as important components of kiwifruit allergy (8, 9). Although we did not measure them, patients with a negative specific IgE response to Act d 8 in our study may have a positive specific IgE response to Act d 12 or Act d 13. In recent

years, storage proteins, such as Ara h 2 in peanuts, have attracted a great deal of attention (10). Therefore, further studies are needed to identify the role of such kiwifruit components.

There were some limitations to our study. First, it was limited by its retrospective nature. Therefore, we do not know whether patients had pollinosis. We also do not know whether they had eaten kiwifruit for the first time when they had allergic symptoms. Moreover, we did not perform an OFC. Therefore, we do not know the patients' symptoms in an OFC or their treatment for such symptoms. Lastly, we did not measure specific IgE response to Act d 1 because the test was not available in Japan.

In conclusion, we investigated the clinical features of pediatric kiwifruit allergy, and validated the performance of specific IgE response to alder, Bet v 1, and Act d 8 for detecting pediatric severe kiwifruit allergy. Negative specific IgE response to alder, Bet v 1, or Act d 8 was identified as a risk factor for severe symptoms among children sensitized to kiwifruit.

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Figure legends

Figure 1. Receiver operating characteristic curve analysis for systemic reaction.

Optimal cutoff point was calculated based on the Youden index.

Abbreviations: AUC, area under the curve; IgE, immunoglobulin E.

Figure S1. Flow diagram of the study subjects.

The study included 54 children. Kiwifruit allergy was defined as immediate symptoms, such as urticaria or oral itching, and specific immunoglobulin E response to kiwifruit ≥ 0.34 kU/L.

Table 1. Characteristics of the 2 groups

			Local reaction (n = 31)	Systemic reaction (n = 23)	P value
Male:female			18:13	15:8	ns ^a
Age at onset, y			7 (2–13)	4 (1–8)	0.0007 ^b
Age at blood exam, y			8 (2–14)	4 (1–9)	0.0008 ^b
Associated atopic disorders	Bronchial asthma		9 (29)	8 (35)	ns ^a
	Atopic dermatitis		23 (74)	15 (65)	ns ^a
	Allergic rhinitis		17 (55)	9 (39)	ns ^a
	Allergic conjunctivitis		12 (39)	5 (22)	ns ^c
	Food allergy	Except kiwifruit	27 (87)	18 (78)	ns ^c
		Vegetable or fruit	15 (48)	5 (22)	ns ^c
Specific IgE level, kU/L	Kiwifruit		2.3 (0.40 to >100)	4.1 (0.50 to 40)	ns ^b
	Alder		3.2 (<0.10 to >100)	0.4 (<0.10 to >100)	0.0020 ^b
	Bet v 1		2.7 (<0.10 to >100)	<0.10 (<0.10 to >100)	0.0002 ^b
	Act d 8		0.52 (<0.10 to >100)	<0.10 (<0.10 to 34)	<0.0001 ^b

Data are presented as median (range) or n (%), as appropriate.

^aAccording to the Chi-square test.

^bAccording to the Mann–Whitney *U* test.

^cAccording to Fisher's exact test.

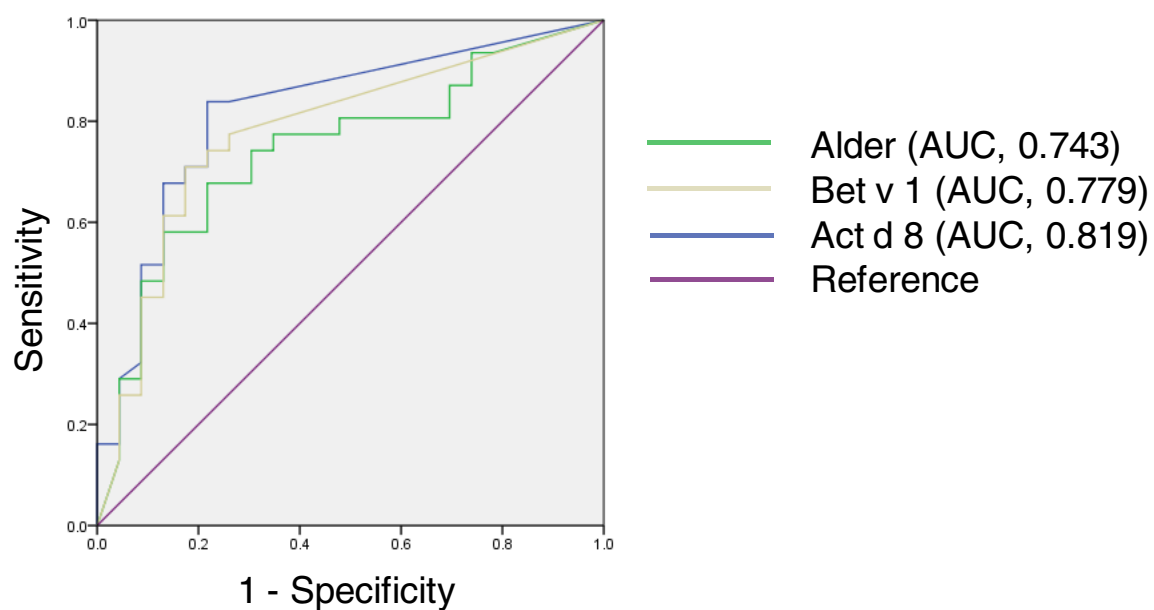
Abbreviations: IgE, immunoglobulin E; ns, not significant.

Table 2. Uni- and multivariate analyses for specific IgE response associated with systemic reaction

Specific IgE	Crude OR (95% CI)	P value	OR adjusted for age at blood exam (95% CI)	P value
Alder	7.6 (2.2–26)	0.001	4.3 (1.1–17)	0.034
Bet v 1	8.1 (2.4–28)	0.003	4.6 (1.2–18)	0.026
Act d 8	19 (4.7–74)	<0.001	12 (2.9–52)	0.001

Positive specific IgE response with the optimal cutoff point was used as the reference.

Abbreviations: CI, confidence interval; IgE, immunoglobulin E; OR, odds ratio.



Specific IgE	Optimal cutoff point, kU/L	Sensitivity, %	Specificity, %
Alder	1.06	67.7	78.3
Bet v 1	0.138	71.0	82.6
Act d 8	0.106	83.9	78.3

Figure 1