

Letter to the Editor

Clinical relevance of IgE to recombinant Gly m 4 in the diagnosis of adult soybean allergy

To the Editor:

Soybean is one of the most important foods causing food allergy in childhood.¹ In addition, studies have shown that allergy to soybean can be caused by IgE cross-reactivity between Bet v 1, a major allergen of birch pollen, and its homologue pathogenesis-related class 10 protein in soybean, Gly m 4.^{2,3} However, the clinical relevance of measuring the level of IgE antibody (IgE-ab) to recombinant Gly m 4 (rGly m 4) is still debated, considering that the concentration of Gly m 4 in soybean extract is quite low and that a considerable proportion of the patients allergic to birch pollen have IgE-ab to Gly m 4 without reporting any symptoms to soybean.^{2,4} Sensitization to the soybean storage proteins Gly m 5 and Gly m 6 have been shown to contribute to more severe soybean allergy.^{1,5} Data from studies on soybean allergy outside Europe, particularly in Asian populations, are still limited.

Japan has one of the largest soybean-consuming populations in the world and tofu, miso, and natto are common soybean foods.⁶ Soybean allergy is the fourth most common cause of food allergy in Japan among infants but, although reported, has been thought until recently to be rare in adults.^{7,8}

The aim of this study was to elucidate the characteristics of soybean allergy among adults and the impact of sensitization to Gly m 4 on soybean allergy and to analyze the diagnostic efficiency of measuring the level of IgE-ab to Gly m 4 in patients with soybean allergy compared with Betulaceae-pollen-sensitized patients without soybean allergy.

We recruited 21 consecutive adult patients with soybean allergy who visited our clinic during 2009. Soybean allergy was diagnosed on the basis of a convincing case history and results of positive skin prick test to soybean extract (extract named "edamame," Torii, Tokyo, Japan) and/or soymilk (Luckme Yakult, Tokyo, Japan). The control group consisted of 93 alder-pollen-sensitized allergic rhinitis patients without soybean allergy recruited from the outpatient clinic during the same study period. Details of the criteria for both patient groups are shown in this article's Online Repository at www.jacionline.org.

The IgE-ab levels to alder pollen, birch pollen, soybean extracts, recombinant Bet v 1 (rBet v 1), rGly m 4, native Gly m 5 (nGly m 5), and native Gly m 6 (nGly m 6) were determined by using the commercially available ImmunoCAP system (Phadia, Uppsala, Sweden). IgE-ab level of 0.35 kU_A/L or more was regarded as positive. IgE-ab levels to nGly m 5 and nGly m 6 were measured in the soybean-allergic group only.

Statistical testing was performed by using the Fisher exact test for the categorical variables and Mann-Whitney *U* test for the continuous variables. The receiver-operating characteristic curves were plotted for IgE-ab levels to rGly m 4 and soybean extract separately and the area under the receiver-operating characteristic curve was calculated and Student's *t* test was conducted for comparison (SPSS ver. 19.0, IBM, Tokyo, Japan). A *P* value of <.05 was considered significant.

The most frequent symptom among the soybean-allergic patients (see Table E1 in this article's Online Repository at www.jacionline.org) was oral allergy syndrome (17 of 21, 81%),

followed by cough/dyspnea (10 of 21, 48%) and abdominal pain/diarrhea (8 of 21, 38%). Soymilk was the most provoking allergen in relation to exposure. Fifteen (71%) of the 21 patients reported reaction to any of the moderately heated soy products such as tofu and boiled edamame, and none to the fermented soy products such as natto, miso, and soy sauce (see Table E2 in this article's Online Repository at www.jacionline.org).

The frequency of sensitization to soybean in soybean-allergic patients was relatively low (48%), whereas all the soybean-allergic patients were sensitized to rGly m 4 (Table I). Only one patient was sensitized to the soybean storage proteins nGly m 5 and nGly m 6.

IgE-ab levels to rGly m 4, as well as to alder pollen, birch pollen, and rBet v 1, were significantly higher in the soybean-allergic group than in the control group (median levels of IgE-ab to rGly m 4, 15.0 vs 0.68 kU_A/L; alder, 48.4 vs 4.77; birch, 42.4 vs 6.49; rBet v 1, 48.4 vs 3.85 kU_A/L; Fig 1, A). The receiver-operating characteristic curve showed that the area under the curve for IgE-ab levels to rGly m 4 was 0.87 (Fig 1, B), which was significantly higher than that for soybean (0.59; *P* < .01). Applying a 4.0-kU_A/L cutoff level, the diagnostic efficiency of rGly m 4 reached 81% and 78%, respectively, in terms of sensitivity and specificity.

This study revealed the characteristics of adult soybean allergy and the impact of sensitization to Gly m 4 in a Japanese population. In Japan, alder pollen (alder is part of the Betulaceae family) is of minor clinical importance in terms of respiratory sensitization, compared with Japanese cedar pollen. However, although sensitization to alder pollen was not an inclusion criterion for the soybean-allergic patient group, all the patients were sensitized to alder pollen and rGly m 4. Moreover, IgE-ab levels to rGly m 4 were markedly higher in the soybean-allergic patients than in the alder-pollen-sensitized control patients. These findings highlight the clinical impact of respiratory sensitization to pollen-derived pathogenesis-related class 10 protein on the development of adult soybean allergy.

The most frequent symptom in the soybean-allergic patients was oral allergy syndrome. Soymilk was the allergen that most prevalently induced symptoms in those who had been exposed, whereas none of the soybean-allergic patients reacted to fermented soybean products. This finding was in agreement with that of Mittag et al.² The explanation is that Gly m 4 is somewhat heat-labile but also apparently susceptible to degradation by fermentation.

This study was carried out in a population with high soybean consumption. Indeed, a study in Japan has shown that all the pediatric patients with soybean allergy were sensitized to Gly m 5 and Gly m 6, and sensitization to these allergens contributes to more severe soybean allergy.⁵ However, only 1 of the 21 adult patients in our study was sensitized to Gly m 5 and Gly m 6, suggesting that primary sensitization to soybean (ie, the oral route) was relatively rare. Therefore, we consider that sensitization to Gly m 4 is of more importance for adult patients with soybean allergy. Moreover, it is important to highlight the finding that some of these Gly m 4-mediated soybean-allergic adult patients experienced severe allergic symptoms including anaphylaxis.

One possible limitation of this study is related to the diagnosis of soybean allergy. Soybean allergy was diagnosed on the basis of

TABLE I. Demographics and frequencies of sensitization to allergens in study subjects

	Soybean-allergic patients (n = 21)	Alder-pollen-sensitized control patients (n = 93)	P value
Sex (female), n (%)	17 (81)	57 (61)	.128
Age (y), mean $\pm$ SD	41.4 $\pm$ 16.1	44.5 $\pm$ 16.6	.438
Comorbidity, n (%)			
Allergic rhinitis	21 (100)	93 (100)	1.000
Asthma	3 (14)	31 (33)	.449
Reported food allergy to soybean, n (%)	21 (100)	0 (0)	<.001
Reported OAS due to any other plant foods,* n (%)	18 (86)	17 (18)	<.001
Sensitization determined by CAP-FEIA (≥ 0.35 kUA/L), n (%)			
Alder pollen	21 (100)	93 (100)	1.000
Birch pollen	21 (100)	93 (100)	1.000
rBet v 1	21 (100)	66 (71)	.003
Soybean	10 (48)	30 (32)	.211
rGly m 4	21 (100)	53 (57)	<.001
nGly m 5	1 (5)	ND	
nGly m 6	1 (5)	ND	

ND, Not done; OAS, oral allergy syndrome.

*Defined as reporting OAS due to any plant foods other than soybean in the structured questionnaire.

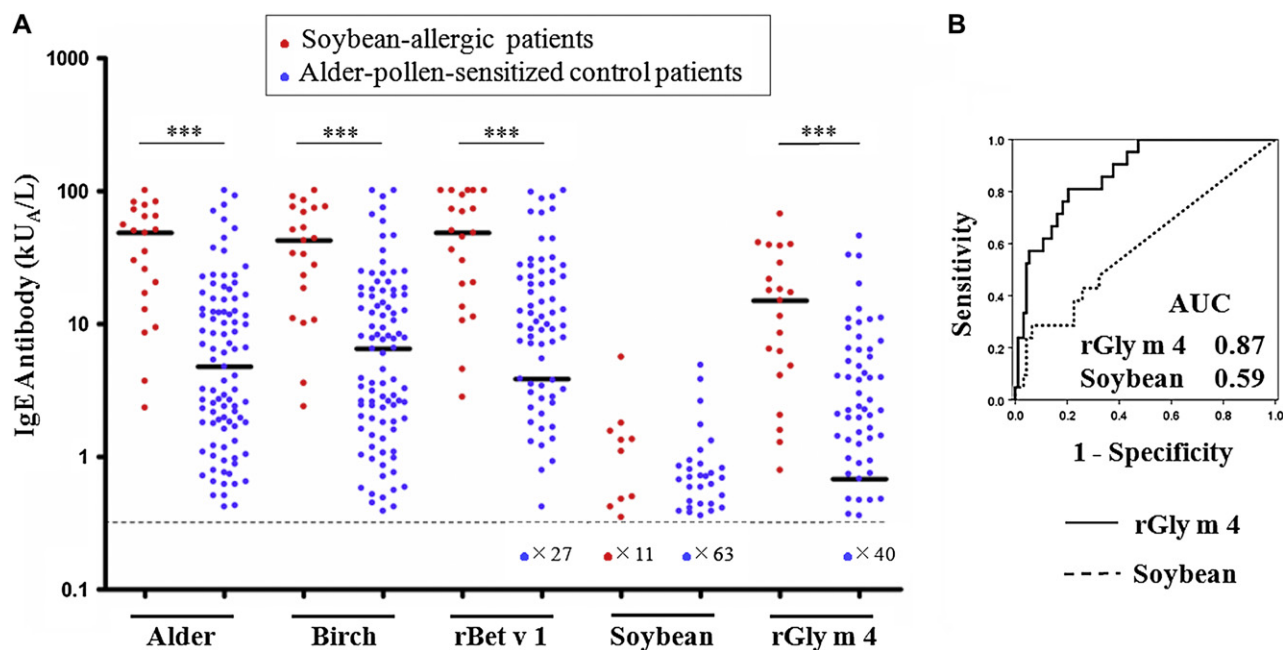


FIG 1. A, Levels of IgE-ab to alder pollen, birch pollen, recombinant Bet v 1 (rBet v1), soybean, and rGly m 4 in sera from soybean-allergic patients and alder-pollen-sensitized control patients. B, Receiver-operating characteristic curve for levels of IgE-ab to soybean and rGly m 4 in the diagnosis of soybean allergy. The number of negative test results in each set of measurement is indicated below the dotted cutoff line (0.35 kUA/L). *** $P < .001$. AUC, Area under the curve.

a convincing case history and positive results of skin prick test. A double-blind placebo-control food challenge was not performed. However, regarding oral symptoms, subjective symptoms are relatively reliable because they occur immediately after the ingestion of specific foods. Recently, Skypala et al⁹ have shown the validity of self-completed structured questionnaire, namely, subjective allergic symptoms, as the basis of the diagnosis of oral allergy syndrome.

We conclude that the discrimination between patients with pollen-related soybean allergy and Betulaceae-pollen-sensitized patients was effectively achieved in this Japanese adult population

with a low prevalence of Betulaceae pollen allergy and high soybean consumption, by measuring the IgE-ab level to rGly m 4. A high level of IgE-ab to rGly m 4 was associated with adult soybean allergy.

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METHODS

Study design

Soybean-allergic patients. We recruited 21 consecutive adult patients with soybean allergy who visited the food allergy department of Sagami National Hospital from January to December 2009. Out of the 21 patients, 11 patients visited the hospital for ongoing disease management whereas 10 were first-time patients visiting the hospital during the study period. Basically, soybean allergy was diagnosed on the basis of case history and results of positive skin prick test to soybean extract (extract named “edamame,” Torii, Tokyo, Japan) and/or soymilk (aqueous soymilk drink, Luckme Yakult, Tokyo, Japan). Edamame is green, immature soybeans and is commonly eaten in Japan after 3 to 4 minutes of boiling. However, regarding 3 patients (patients 8, 9, and 10 in Table E1), we did not have skin prick test data and diagnosed them as having soybean allergy on the basis of their apparent case histories. This study was approved by the Ethics Committee of Sagami National Hospital. Written informed consent was given by the patients prior to enrolment.

Alder-pollen-sensitized control patients. Sensitization to Betulaceae pollen was assessed on the basis of the sensitization to alder pollen, which is the most common pollen from the Betulaceae family in this region. High correlations between IgE to alder pollen and that to birch pollen were described previously.^{E1}

To enroll control subjects representing Betulaceae-pollen-sensitized respiratory-allergic outpatients commonly encountered in our daily practice, we prospectively screened 2 outpatients groups for possible recruitment to the

control group, namely, (1) all the patients who visited the otolaryngology department of the same hospital for the treatment of nasal allergy from January to December 2009 and underwent CAP-IgE to alder pollen for clinical purpose and (2) all the first-time patients who visited the pulmonology department of the same hospital for the treatment of asthma or respiratory allergic symptoms and underwent IgE testing to alder pollen. Before the screening, they were asked to answer a structured questionnaire about their food allergies. This questionnaire included questions about oral hypersensitivity reactions to soybean and other plant foods, shrimp, crab, fish, cuttlefish, or octopus; and self-administered food allergic symptoms other than oral symptoms.

The inclusion criteria for the control group were as follows: (1) an IgE-Ab level of 0.35 kUA/L or more to alder pollen, (2) having nasal/respiratory allergic symptoms during the pollen season, and (3) not reporting soybean allergy in the questionnaire.

Consequently, we screened 212 outpatients who visited the otolaryngology department and 269 patients who visited the pulmonology department. This resulted in the identification of 105 candidate patients for recruitment to the control group (56 patients from the outpatients of the otolaryngology department and 49 patients from the outpatients of the pulmonology department). Among them, 93 patients agreed to participate in this study as control patients.

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TABLE E1. Clinical characteristics, results of skin prick tests, and IgE reactivities to allergens in soybean-allergic patients

Patient no.	Age (y)/sex	Onset age (y)*	Reported symptoms of allergy to soybean	IgE	Skin prick test (mm)		CAP (kUA/L)						Reported food allergies to other plant food†	
					Soybean	Soymilk	Alder	Birch	Soybean	rBet v 1	rGly m 4	nGly m 5		nGly m 6
1	18/F	7	OAS, COU, DYS, AE	1560	4	3	78.3	85.0	1.79	>100	67.1	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Cu, Me, Eg, Ce, Ca, To, Ki, Pi, Or, Ba
2	68/F	68	COU, DYS, AE, OAS, RED	163	-	3	25.7	27.7	<0.35	29.9	8.52	<0.35	<0.35	Ap, Pc, Pr, St, To, Ki, Ba, peanuts
3	70/F	70	NA, DYS, OAS	62	2	2	8.55	10.1	<0.35	10.6	6.48	<0.35	<0.35	-
4	57/F	56	OAS, DYA	25	3	7	2.33	2.38	<0.35	2.82	1.28	<0.35	<0.35	-
5	30/F	30	COU, OAS	236	1	3	64.3	69.2	1.10	93.5	41.0	<0.35	<0.35	Ap, Pc, Ch, Pr, Cu, Me, To, Ki
6	65/M	61	RED, AE, DYS	299	1	3	30.0	33.8	<0.35	45.2	2.05	<0.35	<0.35	-
7	31/M	28	OAS	1820	-	1	17.0	18.5	<0.35	19.9	4.83	<0.35	<0.35	Ap, Ch
8	30/F	29	OAS, COU	122	ND	ND	3.71	3.58	<0.35	4.55	0.79	<0.35	<0.35	Pr, Ce, Ki
9	53/F	47	OAS	1700	ND	ND	>100	>100	0.42	>100	21.7	<0.35	<0.35	Pc, Me, Ki, cashew nuts, Macadamia nuts
10	38/M	35	OAS	200	ND	ND	51.1	53.1	0.50	70.0	17.2	<0.35	<0.35	Ap
11	36/F	22	OAS	655	6	10	64.7	74.2	<0.35	73.2	39.1	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Cu, Me, To, Ki, Or, Ba, hazelnuts
12	47/F	45	OAS, COU, DYS, AE	303	5	ND	48.4	42.4	<0.35	50.4	11.4	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Me, Ce, To, Ki, Pi, Or, Ba
13	41/F	40	ABD, DYA	677	3	5	20.5	23.1	0.35	20.4	6.21	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Cu, Me, Eg, Ce, Ca, To, Ki, Pi, Or, Ba
14	60/F	57	OAS, DYA	391	6	3	50.1	43.9	<0.35	48.4	28.7	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Me, To, Ki, Pi, Ba
15	49/F	48	COU	222	1	6	72.4	75.9	1.33	>100	38.6	<0.35	<0.35	Ap, Pc, Ch, Ki, almond
16	30/F	29	OAS, ABD, DYA	317	5	7	83.2	76.2	5.62	>100	15.0	1.84	2.45	Ap, Pc, Ch, Pr, St
17	17/F	12	ABD, DYA	6030	4	7	82.6	90.8	1.56	>100	39.6	<0.35	<0.35	Pc, Pr, Cu, Me, To, hazelnuts, almond
18	24/F	18	OAS, AE, DYS, DYA	686	2	4	12.8	11.0	0.48	11.3	4.09	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Cu, Me, Eg, To, Ki, Pi, Or, Ba
19	31/M	29	OAS, ABD	878	3	ND	55.6	51.0	<0.35	73.0	18.2	<0.35	<0.35	Ap, Pc, Pr, Me, Pi, Or
20	28/F	27	OAS	489	2	ND	35.0	33.4	1.35	36.1	17.8	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Cu, Me, Ce, Ba, To, hazelnuts
21	47/F	46	OAS, AE, COU, DYS, DYA	91	4	ND	9.39	10.7	<0.35	13.4	1.58	<0.35	<0.35	Ap, Pc, Ch, Pr, St, Me, Ce, Ca, To

ABD, Abdominal pain; AD, atopic dermatitis; AE, angioedema; AN, anaphylaxis; COU, cough; DYA, diarrhea; DYS, dyspnea; F, female; M, male; NA, nasal symptoms; -, Negative; ND, not done; OAS, oral allergy syndrome; RED, redness.

*The age when they first experienced allergic symptoms to soybean.

†Only the important plants are shown. Ap, Apple; Pc, peach; Ch, cherry; Pr, pear; St, strawberry; Cu, cucumber; Me, melon; Eg, eggplant; Ce, celery; Ca, carrot; To, tomato; Ki, kiwi; Pi, pine; Or, orange; Ba, banana.

TABLE E2. Number of episodes and reported reactions to various soybean products in soybean-allergic patients

Patient no.	No. of episodes of soybean allergy	Reported reaction to soybean products				
		Soymilk	Tofu	Edamame	Soybean sprout	Fermented soybean products*
1	>5	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
2	2	Sensitive	Sensitive	Tolerant	Tolerant	Tolerant
3	1	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
4	>5	Never tried	Sensitive	Avoided	Sensitive	Tolerant
5	>5	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
6	3	Sensitive	Sensitive	Tolerant	Tolerant	Tolerant
7	3	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
8	3	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
9	2	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
10	4	Never tried	Sensitive	Tolerant	Sensitive	Tolerant
11	>5	Never tried	Sensitive	Tolerant	Tolerant	Tolerant
12	>5	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
13	2	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
14	2	Sensitive	Sensitive	Tolerant	Tolerant	Tolerant
15	2	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
16	>5	Never tried	Sensitive	Tolerant	Tolerant	Tolerant
17	>5	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
18	>5	Sensitive	Sensitive	Sensitive	Sensitive	Tolerant
19	>5	Never tried	Sensitive	Sensitive	Sensitive	Tolerant
20	2	Sensitive	Tolerant	Tolerant	Tolerant	Tolerant
21	3	Never tried	Sensitive	Sensitive	Sensitive	Tolerant

*Soybean products such as natto, miso, and soy sauce.