

Clinical Features of Four Cases with Cashew Nut Allergy and Cross-Reactivity between Cashew Nut and Pistachio

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ABSTRACT

Background: Few cases of cashew nut (CN) allergy have been reported in Japan. We evaluated the clinical features of 4 cases with CN allergy and investigated the allergens involved.

Methods: In order to investigate the cross-reactivity between CN and pistachios, we performed ImmunoCAP inhibition tests using sera of 4 cases with positive histories of CN allergy and positive results of specific IgE measurement (ImmunoCAP) and skin prick tests. Furthermore, we analyzed the molecular weights of allergens of CN and pistachios by IgE-immunoblotting.

Results: Of the 4 cases (male : female = 1 : 3), there were 3 cases (patient #2-4) and 1 case (patient #1) of anaphylaxis and oral allergy syndrome, respectively. The initial symptom was an oropharyngeal symptom in 3 of the 4 cases, of which 2 cases developed anaphylaxis within 10 minutes after eating only a few pieces of CN. All 4 cases reacted positively to the skin prick test with CN, although 1 case of anaphylaxis tested negatively for CN by ImmunoCAP. Additionally, in 2 cases, IgE-binding to CN and pistachio were inhibited with both pistachios and CN, indicating cross-reactivity between CN and pistachios. IgE-immunoblotting of CN using sera from the 4 cases revealed 2 bands at molecular weights of approximately 33 kd and 42 kd, whereas that of pistachios showed a single band at 36 kd. However, IgE in all 4 sera did not bind to rAna o 2.

Conclusions: In CN allergy, a small amount of CN could induce a severe anaphylactic reaction. Moreover, in cases of suspected CN allergy, reactions to not only CN but also pistachio, which could be cross-reactive to CN, should be examined.

KEY WORDS

allergen, anaphylaxis, cashew nut allergy, cross-reactivity, pistachios

INTRODUCTION

Cashew nuts are tree nuts that belong to *Anacardiaceae*, the same family as pistachio, mango, and ginkgo nuts. Recently, in addition to peanut allergy, allergies to tree nuts such as cashew nuts, walnuts, almonds, pistachios, hazel nuts, and pecan nuts have gained much attention in the United States and Europe due to the high risk of inducing severe anaphylactic reactions. However, very few cases of tree nut allergies have been reported in Japan.

In this study, we have summarized the clinical features of cashew nut allergy observed in 4 cases. We also investigated the cross-reactivity of these cases with pistachios, and examined the allergens involved in cashew nut allergy.

METHODS

SUBJECTS

The subjects of our study were 4 patients (1 male and 3 female) who were seen at our hospital between July 2000 and July 2005 and under suggested close obser-

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Table 1 Clinical features of 4 cases with cashew nut allergy

Case No.	1	2	3	4
Age/Sex	26/F	55/M	12/F	24/F
Diagnosis	Oral allergy syndrome	Anaphylaxis (shock)	Anaphylaxis	Anaphylaxis
Symptoms				
Oropharyngeal symptoms	+	—	+	+
Generalized urticaria	—	+	+	+
Dyspnea	—	+	+	+
Loss of consciousness	—	+	—	—
The initial symptom	Oropharyngeal symptom	Urticaria	Oropharyngeal symptom	Oropharyngeal symptom
Interval between ingestion and the onset	Immediately	10 min	Immediately	30 min
Amount of CN ingested at episode	1 piece	20 pieces	2 pieces	1 piece
Other factors	—	Bathing	Inside the plane	Alcohol
Past history				
Pollinosis	—	—	+	+
Atopic dermatitis	+	—	—	—
Other food allergy	Mango?	—	—	Almond

CN, cashew nut.

vation for possible cashew nut allergy, based on a medical interview. The patients' ages ranged from 12 to 55 years with an average of 29 years.^{1,2}

The following tests were carried out upon obtaining written informed consent from the patients.

LABORATORY TESTS

Serum total IgE and specific IgE levels (ImmunoCAP; Phadia, Uppsala, Sweden) for cashew nuts and pistachios were measured.

SKIN PRICK TEST

To prepare test samples for the skin prick test, we ground individual test nuts (1 g) in a mortar, added them to 2 ml saline solution, and stirred the mixture to obtain a supernatant. The responses to the prick test were determined as negative when the average wheal diameter was equal to that of a negative control, or as false positive (+), positive (2+), strongly positive (3+), and most positive (4+) when the average wheal diameter was more than 25%, 50%, 100%, and 200%, respectively, of a positive control induced by histamine.³

EXTRACTS OF CASHEW NUT AND PISTACHIO

Powdered cashew nuts and powdered pistachios were mixed in 100 ml of cooling diacetone alcohol, and 100 ml of cooling acetone was then added to the mixture. The precipitation mixed with acetone was filtrated by aspiration, and dried as the allergen powder. The allergen powder mixed with 0.1 M phosphate buffer and shaken for 2 hours was centrifuged at 12000 rpm. The supernatants filtrated by aspiration were regarded as allergen extracts.

THE IMMUNOCAP INHIBITION ASSAYS

Duplicated samples of the undiluted pool of sera were premixed 2 : 3 (vol/vol) with 5 progressive 1 : 10 dilutions of the inhibitor solution in 0.1 M phosphate buffer. After a 2-hour incubation at room temperature, specific IgE levels to cashew nut and pistachio were determined using the ImmunoCAP system according to the manufacturer's instructions.

IgE-IMMUNOBLOTTING

The cashew nut and pistachio extract (1 mg/ml in phosphate-buffered saline [PBS]), recombinant Ana o 2 (rAna o 2) (Biomay, Vienna, Austria) (100 mg/ml in diluted water), and the molecular weight markers (Daiichi, Tokyo, Japan) were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and analyzed by the Coomassie-blue staining method. Thereafter, the proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Daiichi) in a transfer cell (Daiichi). The transferred PVDF membrane was incubated at 4°C with 2% of the patients' and the control subjects' sera, respectively, overnight after blocking the membrane with skim milk. IgE antibodies bound to the antigen were visualized using peroxidase-labeled anti-human IgE antibodies (KPL, Gaithersburg, MD, USA).

RESULTS

CLINICAL FEATURES

One patient was given a diagnosis of oral allergy syndrome (OAS) while the other 3 patients were given diagnoses of anaphylaxis; and one of whom showed signs of anaphylactic shock. As for clinical features, oropharyngeal symptoms such as tingling or pruritus in the oral cavity were observed in 3 of the 4 patients

Table 2 Results of ImmunoCAP and skin prick test with cashew nuts and other nuts

Case No.	1	2	3	4
Serum total IgE (IU/ml)	2641	985	592	804
ImmunoCAP (UA/ml [class])				
Cashew nut	0.74 (2)	< 0.34 (0)	1.49 (2)	5.38 (3)
Pistachio	0.97 (2)	< 0.34 (0)	2.60 (2)	3.55 (3)
Peanut	0.90 (2)	< 0.34 (0)	ND	ND
Almond	0.55 (1)	< 0.34 (0)	ND	< 0.34 (0)
Skin prick test				
Cashew nut	4+	4+	4+	4+
Pistachio	ND	3+	4+	4+
Peanut	—	—	—	—
Almond	—	—	—	1+

ND, not done.

(Table 1). Generalized urticaria was found in 3 patients. Initial symptoms occurred mostly in the oral cavity in 3 of the patients and the onset was immediate in 2 patients, within 10 minutes in 1 patient, and within 30 minutes in 1 patient after the ingestion of cashew nuts. The amount ingested needed to induce the allergy was only 1–2 nuts in 3 patients. Patient #2, who consumed 20 cashew nuts, developed anaphylactic shock.

Concerning patients' reaction to other tree nuts or peanuts, patient #4 had clinically experienced tingling in the pharynx and edema of the face when eating almonds.

SKIN PRICK TEST AND MEASUREMENT OF SPECIFIC IGE

In patients #1, #3, and #4, specific IgEs measured using the ImmunoCAP method were classified as Class 2 for both cashew nut and pistachio (Table 2). In the prick test, results for all patients was 4+ to cashew nuts, and 3 patients scored 4+ or 3+ to pistachios. In specific IgE measurements or prick tests against other tree nuts or peanuts, all patients revealed negative results with an exception of specific IgE against peanuts (in class 2) in patient #1, who was able to eat peanuts without any symptoms.

INHIBITION TEST BY THE IMMUNOCAP METHOD

The ImmunoCAP inhibition test was carried out with sera of patients #3 and #4 who showed positive responses to both cashew nuts and pistachios in ImmunoCAP and the skin prick test. In the ImmunoCAP inhibition test using the serum of patient #3, IgE binding to cashew nuts and pistachios was inhibited up to approximately 93.8% and 99.1%, respectively (Fig. 1). In patient #4, while cashew nuts showed 98.2% inhibition of the binding of pistachios, pistachios showed only 40–60% inhibition against cashew nuts (Fig. 2). Taken together, allergen binding to the patients' sera was more efficiently inhibited by

cashew nuts than by pistachios. This suggests the possibility of cross-reactivity caused by sensitization with cashew nuts.

IgE-IMMUNOBLOTTING

As shown in Figure 3, all serum samples from each of the 4 respective patients allergic to cashew nuts which were tested for IgE binding to the crude cashew nut extract and pistachio extract by immunoblotting, showed positive results, whereas no samples from control subjects did. Bands with molecular weight of approximately 33 and 40–42 kd were detected in all 4 sera in immunoblotting with the cashew nut extract, whereas IgE-binding bands at approximately 36 kd were shown by all 4 sera in immunoblotting with the pistachio extract. However, IgE in all 4 sera did not bind to rAna o 2.

DISCUSSION

While reports on allergies to tree nuts are very rare in Japan, many have already been reported in western countries where nut consumption is high.⁴ In particular, physicians are on the alert for patients with cashew nut allergy, since it is more likely to cause anaphylaxis than peanut allergy.⁵ In the United States, 1.1% of the total population is allergic to peanuts or tree nuts and, among tree nuts, cashew nuts are the second most common allergenic food after walnuts.⁴ In a follow-up study performed 5 years after the initial study, conducted through a random phone survey by Sicherer *et al.* in the United States in 1997, approximately 40% of people who had an allergy to tree nuts had experienced allergic symptoms to cashew nuts.⁶ On the other hand, based on our literature search using the "Japana Centra Revuo Medicina" data base, only 5 cases, including the 4 cases in this study, of cashew nut allergy have been reported in the past 10 years (1997 to 2006) in Japan.^{1,2,7} The initial symptoms of allergic reactions after ingestion of cashew nuts occurred mostly in the oral cavity in 4 out of the 5 patients; and 3 patients de-

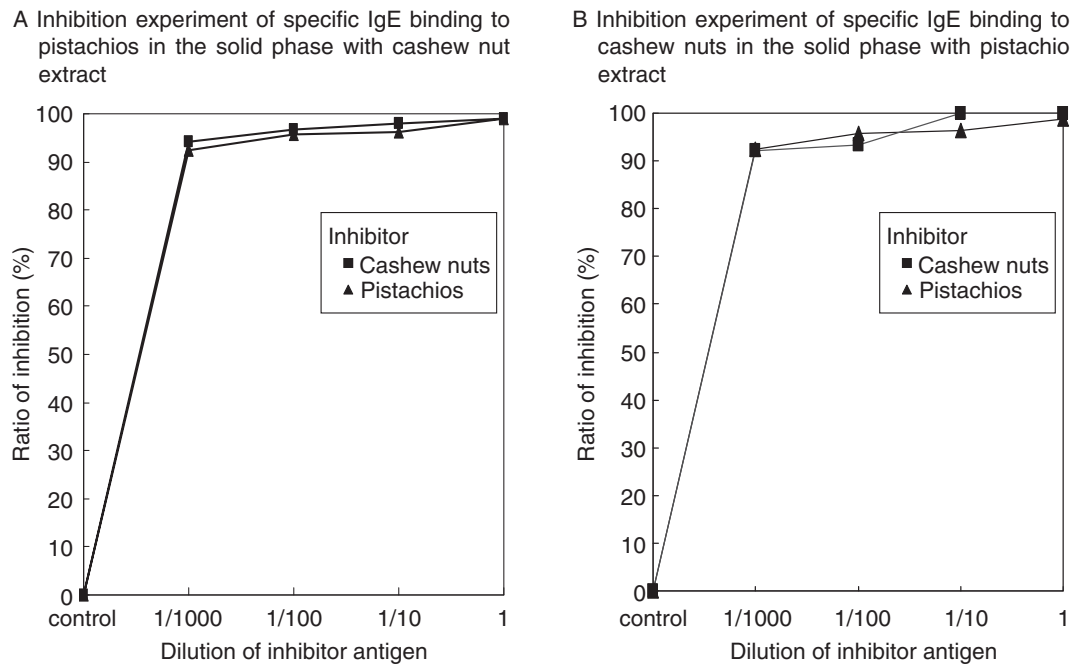


Fig. 1 Inhibition test of case 3 by ImmunoCAP. IgE binding to cashew nuts and pistachios was inhibited up to approximately 93.8% and 99.1%, respectively.

veloped anaphylaxis. Another characteristic of these cases was that even though small amounts of cashew nuts, i.e. only 1–2 nuts, were ingested, it was enough to induce anaphylaxis accompanied by dyspnea.

Table 3 summarizes the clinical features of cashew nut allergy reported in Japanese articles as well as English articles published in the past 10 years that describe the details of the allergic cases.^{8–12} Notable features were that cashew nut allergy was more frequently observed in female adult patients than in males and that many patients had a previous history of allergy diseases such as atopic dermatitis, asthma, and allergic rhinitis. In addition to cashew nuts, many patients were also allergic to other tree nuts such as pistachios, which were the allergenic food for half of the patients, walnuts, almonds, and hazel nuts, in order of decreasing frequency. Regarding clinical symptoms, 20 out of 26 patients in the report by Robotham *et al.* and 6 out of 30 patients in the report by Li *et al.* developed a severe anaphylactic reaction with shock. In summary of the articles that described oropharyngeal symptoms, such symptoms were found in all of the 30 cases in the report by Li *et al.* and in 4 of the 5 cases in Japan; thus, it is notable that cashew nut allergy seems to be accompanied frequently by oropharyngeal symptoms. Careful attention must be paid even in patients who have only oropharyngeal symptoms, because they may develop a severe allergy if they continue to ingest allergenic foods. Regarding testing of these allergy patients, the positive ratio was generally high in specific IgE measurements and also the skin prick test in Japan and other countries. For

example, all 30 patients in the report by Li *et al.*¹⁰ and 2 patients receiving the tests in the report by Fernandez *et al.*¹³ showed positive responses in CAP-RAST, which has been measured by the same system as ImmunoCAP, and the skin prick test. Parra *et al.*¹⁴ performed only the skin prick test, and all 3 patients reacted positively. In particular, the skin prick test showed positive results in almost all the cases reported in Japan and other countries, including the patient #2 in our study (present patient) who was negative in ImmunoCAP. In this study, the negative result in patient #2 in ImmunoCAP may be due to a low concentration or absence of the causative allergen of cashew nuts in the extract used in the test.

For the first time in Japan, our studies have proved the cross-reactivity of cashew nuts and pistachios based on 2 of the 4 present cases using the ImmunoCAP inhibition test. Based on the result of immunoblotting, causative allergens of cashew nuts were suggested to have a molecular weight of 33 kd and 40–42 kd. To identify the allergen involved with cross-reactivity between cashew nuts and pistachios, IgE-immunoblotting was performed using a recombinant allergen, Ana o 2, which has a similar molecular weight to both the 33 kd band of cashew nuts and the 36 kd band of pistachios. However, rAna o 2 did not bind with the sera of any of our patients, suggesting that in the present study the cross-reactive allergen in cashew nut allergy may be one or more proteins other than Ana o 2.

Pistachios and mangos, which belong to *Anacardiaceae*, the same family as cashew nuts, have been re-

Four Cases of Cashew Nut Allergy

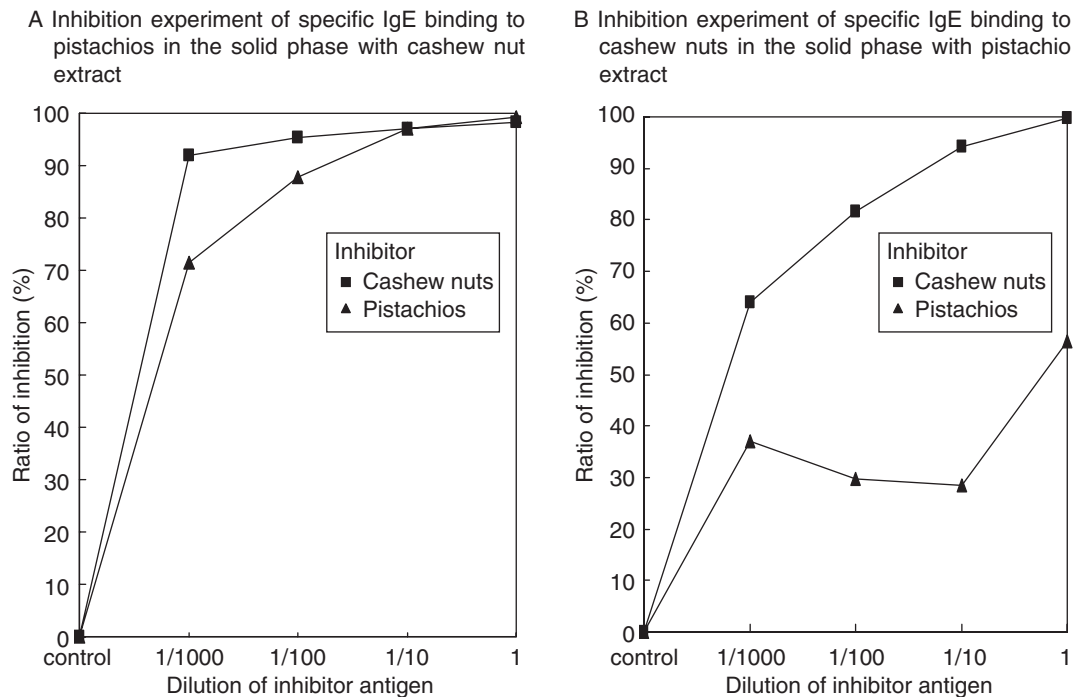


Fig. 2 Inhibition test of case 4 by ImmunoCAP. IgE binding to cashew nut and pistachio was inhibited up to approximately 56.3% and 98.2%, respectively.

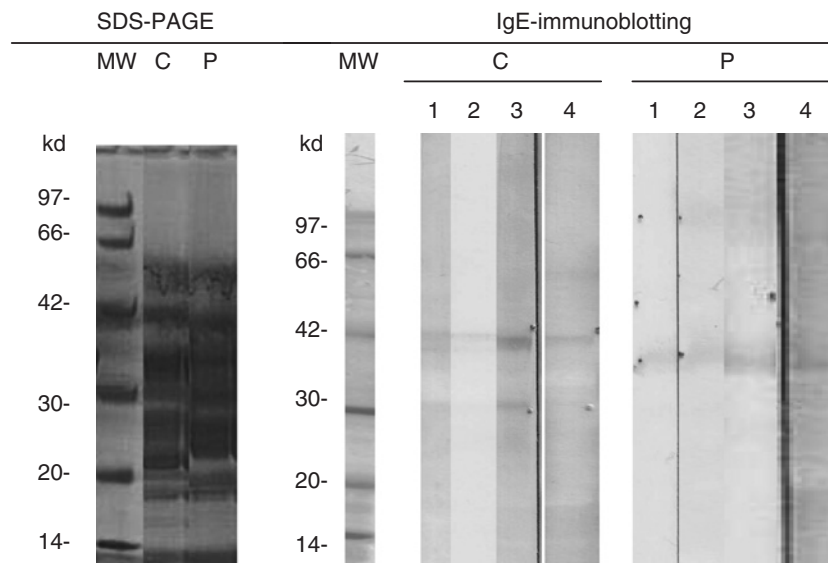


Fig. 3 IgE-immunoblotting with cashew nuts and pistachios. IgE-binding bands at molecular weights of approximately 33 kd and 42 kd were shown in the sera of all four patients in IgE-immunoblotting of cashew nuts, whereas IgE-immunoblotting of pistachios with four patients sera showed a single band at 36 kd. The samples from five control subjects showed negative results in IgE-immunoblotting of cashew nuts and pistachios. MW, molecular weight; C, cashew nuts; P, pistachios; 1–4, Patients # 1–4; respectively.

ported as foods that show cross-reactivity with cashew nuts. In 1999, Quercia *et al.*¹² reported that a male patient who had a history of allergy to pistachios

showed symptoms such as edema of the lips, dyspnea, and vomiting following ingestion of cashew nuts, and showed positive responses to both pistachios and

Table 3 Reports of cashew nut allergy over the recent 10 years (PubMed 1997–2006)

	Number of cases	Age (mean) (y)	Sex (M : F)	Other food allergy		Symptom or severity		Past history	
1 Garcia F	3	1–36 (16)	0 : 3	Pistachio	1/3	Urticaria	3/3	AD	1/3
				Others	1/3	Dyspnea	2/3	AR	1/3
2 Robotham JM	26	2–63 (32)	9 : 17	Pistachio	2/18	Grade* 2	4/26	AD	17/26
				Walnut	6/18	Grade 3	2/26	AR	22/26
				Other TN	12/18	Grade 4	17/26	BA	17/26
						Grade 5	3/26		
3 Li H	30	22–61 (38)	8 : 22			Urticaria	26/30	AR	4/30
						Oral symptom	30/30	BA	4/30
						Abdominal symptom	21/30		
						Dyspnea	13/30		
						Shock	6/30		
4 Rance F	42	1.5–11 (2.7)	31 : 11	Pistachio	28/42	Skin symptom	28/112	AD	25/42
				Walnut	4/42	Dyspnea	63/112	BA	22/42
				Other TN	18/42			AR	8/42
5 Quercia O	1	42	1 : 0	Pistachio		Itching in oral cavity			
				Hazelnut		Itching of lips			
						Dyspnea			
6 Kakemizu N	1	38	0 : 1	Almond		Skin symptom			
						Oropharyngeal symptom			
7 Our cases	4	12–55 (29)	1 : 3	Almond	1/4	Urticaria	3/4	AD	1/4
						Oropharyngeal symptom	3/4	AR	2/4
						Dyspnea	3/4		
						Shock	1/4		

TN, tree nuts; AD, atopic dermatitis; AR, allergic rhinitis; BA, bronchial asthma.

*Clinical severity of anaphylaxis is graded based on the system proposed by Sampson H.A.¹⁸

Table 4 Cashew nut allergens

Allergen name	MW	Biochemical name	Positive ratio of specific IgE	Homologous allergen derived from other foods
Ana o 1	50 kd	Vicilin-like protein	50 %	?
Ana o 2	33 kd	Legumin-like protein (11S-globulin)	62 %	G2 glycinin (Soybean)
Ana o 3	13 kd	2S-albumin family	81 %	Jug r 1 (Walnut)

MW, molecular weight.

cashew nuts in the skin prick test. Furthermore, in 1995, Fernandez *et al.*¹³ and in 1993, Parra *et al.*¹⁴ demonstrated the cross-reactivity of these foods in the CAP-RAST inhibition test. Garcia *et al.*⁸ reported that all 3 patients who developed anaphylaxis against cashew nuts showed positive responses to both cashew nuts and pistachios in the skin prick test, and one of the patients showed allergic symptoms even after ingestion of pistachios. In the present study, all 3 patients who received the skin prick test showed strong positive responses against pistachios and furthermore, in 2 of these patients, cross-reactivity of pistachios and cashew nuts was proved by the CAP-RAST inhibition test.

Until now, Ana o 1, a vicilin-like protein,¹⁵ Ana o 2, a legumin-like protein (11S globulin),¹⁶ and Ana o 3, a

2S albumin,⁹ have been identified as allergens of cashew nuts (Table 4); all of these 3 proteins are biologically classified as seed storage proteins. It has been reported that Ana o 1, Ana o 2, and Ana o 3 are sensitized in 50%, 62%, and 81% of patients with an allergy to cashew nuts, respectively.^{9,15,16} As allergens of pistachios cross-reactive to cashew nuts, proteins with a molecular weight of 15, 30, 34, 41, 52, and 60 kd have been identified. Garcia *et al.* stated that among these proteins, those with a molecular weight of 15, 30, and 60 kds are allergens that showed cross-reactivity with cashew nuts (Table 5).⁸ Ana o 3, with the highest ratio of sensitization in cashew nut allergy among the 3 allergens identified, is highly homologous with an allergen of walnuts, Jug r 1.¹⁶ Robotham *et al.* reported that one-third of 26 patients with

Table 5 Pistachio allergens indicated to be cross-reactive to cashew nut allergens

Author	MW of pistachio allergen presumed	Biochemical name	Cashew nuts allergen suspected homology to pistachio allergen
Garcia F ⁸	15 kd	2S albumin family	→ Ana o 3 ?
	30 kd		
	60 kd		
Parra FM ¹⁴	34 kd		→ Ana o 2 ?
	41 kd		
	52 kd		→ Ana o 1 ?
	60 kd		

MW, molecular weight.

cashew nut allergy were also allergic to walnuts. Other foods that contain 2S albumin include sunflower seeds, peppers, sesame, peanuts, and Brazil nuts.¹⁷

In Japan, in light of the recent diversification in diet, we should be aware of the possibility that the number of allergy cases due to cashew nuts might increase in the near future. Furthermore, it is important to understand that patients with cashew nut allergy should be carefully examined for possible pistachio allergy, which could be developed through the cross-reactivity of cashew nuts and pistachios.

This study was presented in part at the 56th Annual Meeting of the Japanese Society of Allergology.

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