Received: 14 January 2018 | Accepted: 30 April 2018

DOI: 10.1002/cia2.12019

ORIGINAL ARTICLE

WILEY Journal of Cutaneous Immunology and Allergy

Tropomyosin is a minor but distinct allergen in patients with shrimp allergies in Japan

Onon Tsedendorj MD[1] | Yuko Chinuki MD, PhD[1] | Kiyoe Ueda[1] | Kunie Kohno PhD[1] | Atsuko Adachi MD, PhD[2] | Eishin Morita MD, PhD[1]

[1]Department of Dermatology, Shimane University Faculty of Medicine, Izumo, Japan

[2]Department of Dermatology, Hyogo Prefectural Kakogawa Medical Centre, Kakogawa, Japan

Correspondence
Onon Tsedendorj, Department of Dermatology, Shimane University Faculty of Medicine, Izumo, Japan.
Email: onon.tsedendorj@gmail.com

Funding information
The Practical Research Project for Allergy Diseases and Immunology (Research on Allergic Disease and Immunology) from Japan Agency for Medical Research and Development (AMED); The Monbukagakusho Honors Scholarship for Privately-Financed International Student

Abstract

Recently, purified allergens have been utilized for allergen-specific IgE tests, which are highly useful because of their excellent specificity and sensitivity in identifying allergic patients. The purpose of this study was to evaluate the specificity and sensitivity of tropomyosin-specific IgE test in the diagnosis of shrimp allergies in Japan. We enrolled 27 patients with shrimp allergy and five patients with atopic dermatitis, who had no history of allergic reactions to shrimp but showed positive results in tropomyosin-specific IgE test, in this study. Tropomyosin-specific IgE was determined by IgE immunoblotting and tropomyosin-specific IgE test. Involvement of carbohydrate moieties in IgE binding to the allergens was examined by periodate treatment. Tropomyosin-specific IgE was detected in 13 and positive in 10 of the 27 patients with shrimp allergy, whereas shrimp-specific IgE was detected in 21 and positive in 20 of these 27 patients. Of the 13 patients with detectable levels of tropomyosin-specific IgE, seven were confirmed to have tropomyosin-specific IgE by immunoblotting analysis, whereas no IgE binding was seen in the five patients with atopic dermatitis, indicating the high specificity of the tropomyosin-specific IgE test. The level of tropomyosin-specific IgE was well correlated with those of shrimp-specific IgE and Der p 10-specific IgE. Our findings indicated tropomyosin is a minor but distinct allergen in patients with shrimp allergy, especially causing symptoms of OAS.

KEYWORDS
cross-reactivity, crustaceans, shellfish, shrimp, tropomyosin

1 | INTRODUCTION

Shellfish including crustaceans are the most frequent causes of IgE-mediated allergic reactions to food, which range from mild oral allergy syndrome (OAS) to systemic anaphylaxis. Food allergies caused by crustaceans are known to cross-react among the crustacean species, such as shrimp and crab.

The first major allergen identified in crustaceans is tropomyosin, a muscle protein of shrimp (Pen a 1).[1] Following studies have demonstrated that more than 70% of the patients with shrimp

allergy have IgE to tropomyosin.[2,3] Tropomyosin was identified as a major allergen in other crustaceans such as lobster *Homarus americanus* (Hom a 1)[4] and crab *Charybdis feriatus* (Cha f 1),[5] mollusks such as squid *Todarodes pacificus* (Tod p 1),[6] oyster *Crassostrea gigas* (Cra g1),[7] and snail *Turbo cornutus* (Tur c 1).[8] In addition, many reports present tropomyosin to be an important component of immune and allergic reactions in other invertebrates such as house dust mites (HDMs) *Dermatophagoides farinae* (Der f 10),[9] *Dermatophagoides pteronyssinus* (Der p 10),[10] and cockroach *Periplaneta Americana* (Per a 7).[11] Tropomyosins from HDMs have a high sequence homology to shellfish tropomyosins, with an 81% amino acid sequence similarity between shrimp (Pen a 1) and HDMs (Der p 10),[12] and cross-reactivity between tropomyosins from shellfish and HDMs has also been well documented.[13,14] These findings provided evidence for cross-reactivity in allergen-specific IgE test among crustaceans, mollusks, insects, arachnids, and even nematodes. The elucidation of the amino acid sequences of tropomyosin protein in these species is important to understand IgE cross-reactivity among tropomyosins of different species.

Allergen-specific IgE test is widely used in the diagnosis of immediate-type food allergies because this test can be performed with ease for identifying causative allergens in patients with food allergies. It is noteworthy that the in vitro immunoassay system usually employs crude extracts of foodstuffs to detect the food-specific IgE; thus, the sensitivity and specificity of the test are not always satisfactory in identifying patients with true food allergies. Recently, purified allergens have been utilized for allergen-specific IgE tests; these tests show excellent specificity and sensitivity in identifying allergic patients and are termed component-resolved diagnostics.[15-19] The purpose of this study was to evaluate the specificity and sensitivity of tropomyosin-specific IgE test in the diagnosis of shrimp allergies in Japan.

2 | METHODS

2.1 | Subjects

A total of 27 patients with shrimp allergy, who had consulted by the Department of Dermatology in Shimane University Hospital between December 2012 and April 2017, and the Department of Dermatology in Kakogawa Prefectural Medical Center between January 2004 and August 2011, were enrolled in this study. Shrimp allergy was diagnosed according to the history of immediate allergic symptoms after ingesting shrimp, such as black tiger and a positive reaction in the skin prick test using shrimp extract (Torii Pharmaceutical Company, Japan). The patients were classified into three groups according to their clinical symptoms: group I, 11 patients with OAS after ingesting shrimp; group II, 13 patients with urticaria without OAS; and group III, three patients with abdominal pain, asthma, diarrhea, and angioedema without OAS and urticaria. Five patients with atopic dermatitis (AD) who showed positive shrimp-specific IgE test but had no allergic history after shrimp ingestion were enrolled as controls. The background of the subjects is described in Table 1. This study was approved by the ethics committee of the Shimane University Faculty of Medicine (approval No. 469). Informed consent for this study was obtained from all subjects by signing in the informed consent format.

2.2 | Measurement of serum allergen-specific IgE

Sera obtained from the patients were frozen and stored at −20°C until use. Serum shrimp-specific IgE, crab-specific IgE, squid-specific IgE, tropomyosin-specific IgE, Der p 1 (peptidase 1)-specific IgE, and Der p 10 (tropomyosin)-specific IgE levels were detected by the CAP-fluorescent-enzyme immunoassays (CAP-FEIA) (ImmunoCAP®, ThermoFischer Scientific, Uppsala, Sweden). Values greater than 0.35 Ua/mL were considered to be detectable and those greater than 0.70 Ua/mL were considered to be positive.

2.3 | IgE immunoblot analysis

Two grams of commercially available black tiger shrimp *Penaeus monodon* was suspended in 20 mL ice-cold distilled water and homogenized using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The homogenate was transferred into centrifugation tubes and centrifuged at 15 000 *g* for 15 minutes at 4°C, and the supernatant was used as the water-soluble protein. The pellet was dissolved in 2% sodium dodecyl sulfate (SDS) sample buffer, heated for 10 minutes at 80°C, and centrifuged at 15 000 *g* for 15 minutes at 4°C. The supernatant was collected as the water-insoluble proteins. Protein concentrations of the samples were measured using a protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Purified natural shrimp tropomyosin was purchased from Indoor Biotechnologies, Charlottesville, Virginia, USA.

IgE immunoblotting was performed as previously described.[20] Briefly, proteins were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Samples (shrimp extract: 6 μg/lane, purified tropomyosin: 0.5 μg/lane) were loaded on a 12.5% polyacrylamide gel and electrophoresed at 250 V for one hour. The separated proteins were transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Immobilon-P®; Millipore, Billerica, MA, USA) using a semi-dry immunoblot apparatus (Bio-rad) at 25 V for one hour. After the transfer was completed, the membrane was placed in blocking solution (5% skimmed milk in Tris-buffered saline) for one hour and further incubated with patient sera diluted to 5% in blocking solution for overnight at 28°C. The blotted membranes were washed three times with washing solution (20 mmol/L Tris-HCl, pH 8.0; 0.15 mol/L NaCl; 0.1% Tween-20; TBST) and incubated with polyclonal goat antihuman IgE conjugated to horseradish peroxidase (GtxHu-002-EHRPX®, ImmunoReagents, USA), used at a dilution of 1:40 000 in TBST, for one hour. The membranes were then washed three times with TBST and the bound IgE was visualized on RX-U Fuji medical X-ray film® (FUJIFILM Co., Tokyo, Japan) using an enhanced chemiluminescent ECL prime kit® (GE Healthcare UK Ltd, Buckinghamshire, UK). Total protein in the gel was stained with Coomassie Brilliant Blue (CBB). Relative molecular masses of the bands were calculated with Quantity One Precision plus Protein Standards Analysis Software® (Bio-Rad).

TABLE 1 Background of the subjects

Group	Subject No	Age (y)	Gender[a]	Clinical symptom[b]		
				Shrimp	Crab	Squid
I	1	8	F	OAS	OAS	OAS
	2	16	F	OAS	OAS	Discomfort
	3	37	M	OAS	None	Unknown
	4	32	F	OAS, C	Unknown	Unknown
	5	30	F	OAS, AP, F	OAS, AP, F	None
	6	23	M	OAS, AP, R	OAS, AP, R	OAS, AP, R
	7	46	F	OAS, U	Unknown	Unknown
	8	31	F	OAS, U, CU	U	U
	9	20	F	OAS	Unknown	Unknown
	10	31	F	OAS	OAS	OAS
	11	14	M	OAS, U	OAS	OAS
II	12	27	F	U	U	Unknown
	13	16	M	U	U	Unknown
	14	11	F	U, Dyspnea	U, S	None
	15	36	F	U, CU	Unknown	Unknown
	16	24	F	U, RD	Unknown	Unknown
	17	20	M	U, RD, AE	Unknown	Unknown
	18	68	F	U, S	Unknown	Unknown
	19	70	M	U, S	U, S	Unknown
	20	45	M	U, S	Unknown	Unknown
	21	25	M	U, S	Unknown	Unknown
	22	51	F	U	U, AP	U
	23	21	F	U	Unknown	Unknown
	24	17	F	U	Unknown	Unknown
III	25	54	M	Diarrhea	Dyspnea	None
	26	33	F	As	As	As
	27	26	M	AP, AE	Unknown	Unknown
AD[c]	28	13	M	None	None	None
	29	27	M	None	None	None
	30	23	M	None	None	None
	31	46	M	None	None	None
	32	26	M	None	None	None

[a]Gender: M; male, F; female,
[b]Clinical symptom: OAS, oral allergy syndrome; U, urticaria; CU, contact urticaria; C, cough; As, asthma; AE, angioedema; AP, abdominal pain; F, fever; R, respiratory; RD, respiratory discomfort; S, shock.
[c]AD: atopic dermatitis patient who has no history of allergic reaction to shrimp but has positive shrimp-specific IgE test.

2.4 | Sodium periodate treatment

Binding of IgE to carbohydrate moieties of shrimp protein was examined by treating the blotted PVDF membranes with sodium periodate treatment according to a method previously described.[20] Briefly, water-soluble and water-insoluble shrimp proteins were separated on 12.5% SDS-PAGE under reducing condition and electrophoretically transferred to a PVDF membrane. After the blotting, PVDF membranes were incubated with 0.02 mol/L NaIO4 in 0.05 mol/L sodium acetate (pH 5.0) for one hour at 28°C in the darkness. The membranes were then immunoblotted as described above. The blotted PVDF membrane immersed in 0.05 mol/L sodium acetate (pH 5.0) served as the control.

2.5 | Statistical analysis

Levels of allergen-specific IgE were compared using Pearson correlation test and *P*-value with < 0.05 was considered to be significant. Statistical analysis was performed with SPSS Statistical version 24 (IBM Corporation, Tokyo, Japan).

3 | RESULTS

3.1 | Allergen-specific IgE test

Shrimp-specific IgE was detected in 21 (detection rate 77.8%) and positive in 20 (positivity rate 74%) of the 27 patients with shrimp allergy, whereas tropomyosin-specific IgE was detected in 13 (detection rate 48.1%) and positive in 10 (positivity rate 37%) of these 27 patients (Table 2). When the patients were divided into three groups according to their symptoms, the shrimp-specific IgE was detected in 10 (detection rate 91%) and positive in 10 (positivity rate 91%) of the 11 patients in group I, detected in 8 (detection rate 61.5%) and positive in eight (positivity rate 61.5%) of the 13 patients in group II, and detected in three (detection rate 100%) and positive in 2 (positivity rate 67%) of the three patients in group III (Table 2). Tropomyosin-specific IgE was detected in seven (detection rate 64%) and positive in six (positivity rate 54.5%) of the 11 patients in group I, detected in five (detection rate 38.5%) and positive in three (positivity rate 23%) of the 13 patients in group II, and detected in one (detection rate 33.3%) and positive in one (positivity rate 33.3%) in the three patients in group III.

TABLE 2 Summary of the results of total IgE, allergen-specific IgE, and IgE immunoblotting

			Specific IgE (Ua/mL)						Relative band in IgE immunoblotting (kDa)	
Group	Subject No	Total IgE (Ua/mL)	Shrimp	Crab	Squid	Tropomyosin	Der p 1	Der p 10	Soluble	Insoluble
I	1	ND[a]	1.06	1.09	ND	0.53	ND	ND	–[b]	–
	2	1020	10.9	10.2	5.44	8.09	75.7	21.7	34	35
	3	4281	5.66	2.79	<0.35	<0.35	18.4	<0.35	–	–
	4	ND	10.3	9.03	ND	7.64	ND	ND	34	35
	5	ND	1.4	0.98	ND	<0.35	ND	ND	–	–
	6	194	41.4	18.9	7.46	35.9	<0.35	50.5	34	35, 80
	7	ND	<0.35	<0.35	ND	<0.35	ND	ND	–	43
	8	ND	6.2	2.72	ND	<0.35	ND	ND	–	–
	9	26.8	2.76	2.61	0.97	1.86	<0.35	2.45	–	–
	10	451	3.25	2.08	1.02	1.76	5.11	2.81	–	–
	11	1495	64.4	53	16	56	33.9	79.8	34	35
II	12	ND	<0.35	<0.35	<0.35	<0.35	ND	ND	–	–
	13	951	2.22	1.79	<0.35	<0.35	<0.35	<0.35	–	–
	14	ND	<0.35	<0.35	<0.35	<0.35	ND	ND	–	–
	15	ND	<0.35	ND	ND	<0.35	ND	ND	–	–
	16	290	<0.35	<0.35	<0.35	<0.35	6.8	<0.35	–	–
	17	1609	8.69	6.16	0.51	<0.35	7.28	<0.35	–	–
	18	ND	1.88	4.17	ND	0.59	ND	ND	–	–
	19	ND	5.37	9.76	0.7	0.46	ND	ND	150, 200	–
	20	512	2.08	1.63	<0.35	<0.35	29.5	<0.35	–	–
	21	749	4.99	3.3	1.92	2.71	35	3.22	34	35
	22	613	2.61	1.14	<0.35	0.76	2.06	1.4	–	–
	23	423	13.3	7.31	5.76	6.16	4.14	7.58	–	35
	24	1886	<0.35	0.51	<0.35	<0.35	77.5	<0.35	–	–
III	25	ND	7.98	6.23	ND	<0.35	ND	ND	–	–
	26	ND	2.41	2.69	ND	2.22	ND	ND	–	35
	27	1059	0.6	<0.35	<0.35	<0.35	11.3	<0.35	–	–
AD[c]	28	4267	3.04	3.09	1.25	1.02	ND	ND	–	–
	29	25 200	2.03	3.64	0.78	0.46	ND	ND	16, 18	43
	30	4560	3.08	1.04	0.59	<0.35	ND	ND	–	150
	31	9467	5.39	3.53	2.19	0.49	ND	ND	–	–
	32	7320	6.54	4.33	<0.35	<0.35	ND	ND	37	–

[a]ND, not done.
[b]–, negative.
[c]AD, atopic dermatitis patient who has no history of allergic reaction to shrimp but has positive shrimp-specific IgE test.

In the five patients with AD, without any history of allergic reaction to shrimp, tropomyosin-specific IgE test was detected in three subjects (detection rate 60.0%) and positive in one subject (positivity rate 20.0%).

In the positive tests for the 27 patients with shrimp allergy and five patients without shrimp allergy, sensitivity of shrimp-specific IgE test was 74% but specificity was 0%, whereas sensitivity of tropomyosin-specific IgE test was 37% but specificity was 80%.

3.2 | IgE immunoblotting

Serum IgE binding to water-soluble and water-insoluble shrimp proteins was examined by immunoblotting in 27 patients with shrimp allergy and five patients with AD (Figure 1). No common band was detected specific for the patients with shrimp allergy. The immunoblotting showed a band with a molecular weight of 34 or 35 kDa, which corresponded to tropomyosin, in seven (subjects 2, 4, 6, 11, 21, 23 and 26) of the 27 patients with shrimp allergy (Figure 1, Table 2). When serum IgE binding to the water-soluble and water-insoluble proteins of shrimp was examined by immunoblotting, a 34-35 kDa band was detected both with the water-soluble and water-insoluble proteins of shrimp in five patients (subjects 2, 4, 6, 11 and 21), but not detected with the water-soluble protein of shrimp in two patients (subjects 23 and 26). These seven patients also showed a positive tropomyosin-specific IgE test. High-molecular weight proteins of 150 and 200 kDa were found in subject 19 and an additional 80-kDa band was seen in subject 6, suggesting the presence of additional minor allergens in black tiger shrimp. Several bands of various sizes were detected in the patients with AD. However, no clear band corresponding to tropomyosin was seen except in subject 29, who had an IgE-binding band with a molecular weight of 43 kDa.

Serum IgE binding to purified tropomyosin was detected in the seven patients with shrimp allergy (subjects 2, 4, 6, 11, 21, 23, and 26), whereas no binding was seen in the five patients with AD (Figure 2). The binding was well compatible with the binding to 34-35 kDa bands seen with shrimp extract proteins, indicating that these bands predominantly arose from tropomyosin.

3.3 | Sodium periodate treatment

We performed periodate oxidation of sugar residues to determine whether carbohydrate epitopes were involved in the IgE reactivity of shrimp extract. In the subjects 2, 6, 11, 21, and 23, individual bands with molecular weight of 34-35 kDa were not affected. However, the 80-kDa band found in subject 6 and 43-kDa band found in subject 29 was diminished, suggesting IgE binding to carbohydrate moieties in these bands (Figure 3).

3.4 | Correlation among allergen-specific IgE levels

The level of tropomyosin-specific IgE strongly correlated to that of Der p 10-specific IgE, but not to that of Der p 1-specific IgE (Figure 4). The level of tropomyosin-specific IgE strongly correlated to that of shrimp-specific IgE, whereas the level of Der p 10-specific IgE did not correlate to that of Der p 1-specific IgE (Figure 5).

4 | DISCUSSION

In this study, we demonstrated that tropomyosin is a minor but distinct allergen in the patients with shrimp allergy in Japan, because, in

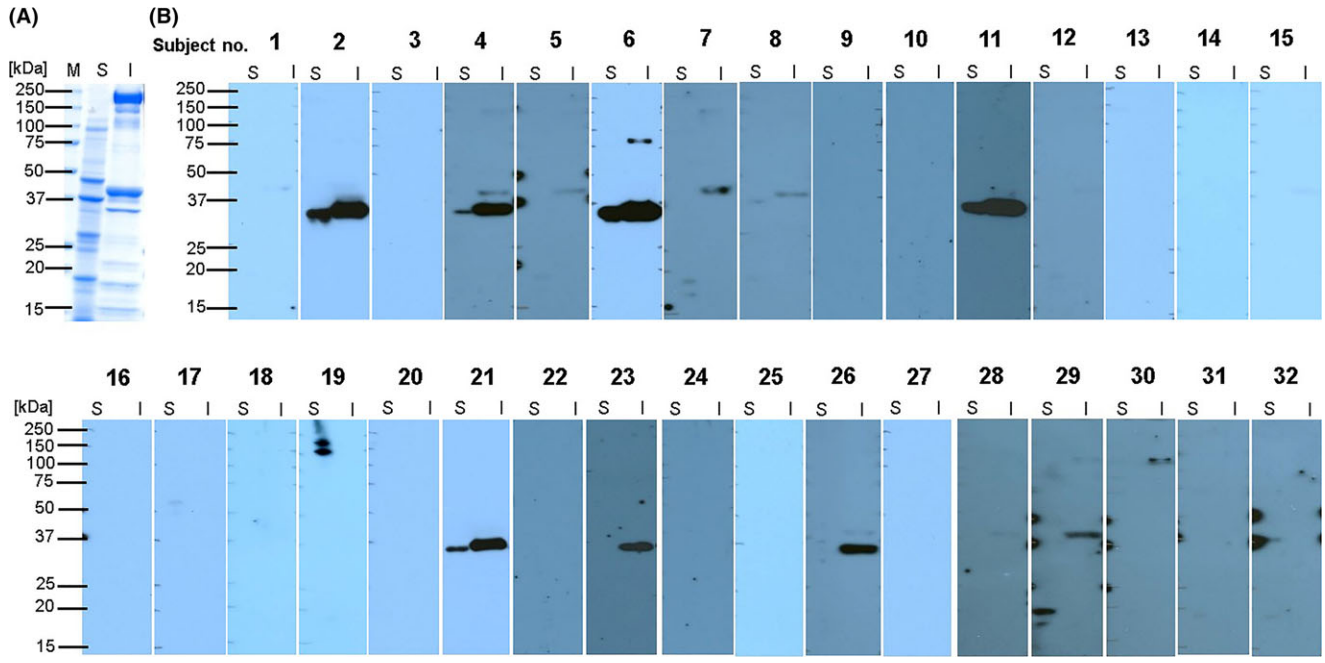

FIGURE 1 IgE immunoblot analysis using water-soluble and water-insoluble shrimp proteins. A, Gel stained with Coomassie Brilliant Blue. B, Immunoblotting with the subjects' sera. Lane M: molecule weight marker proteins; Lane S: water-soluble shrimp proteins; I: water-insoluble shrimp proteins

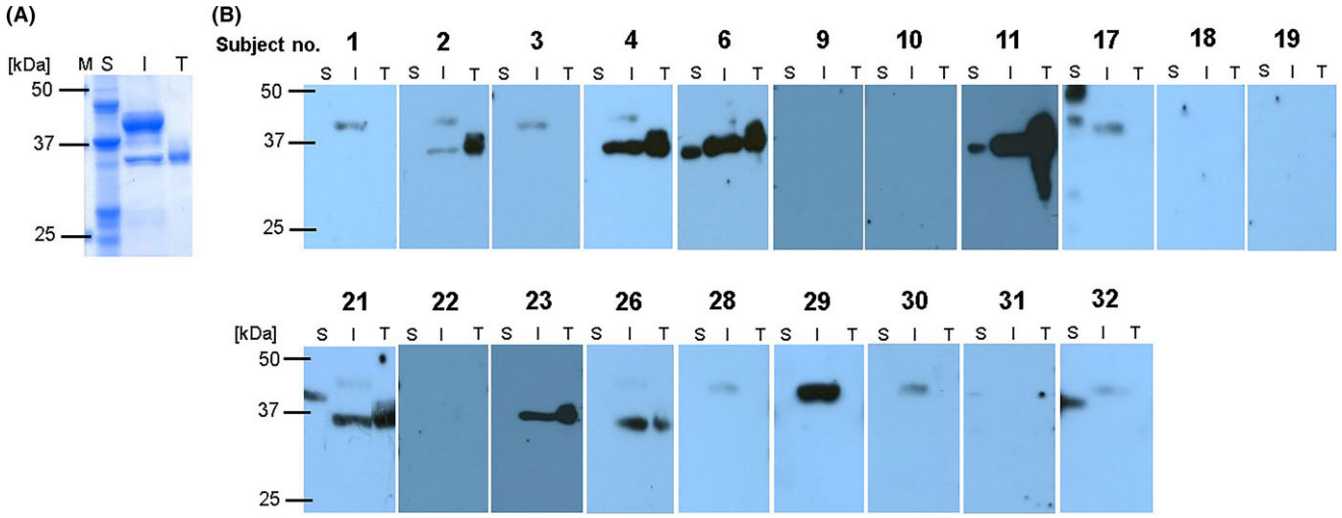

FIGURE 2 IgE immunoblot analysis using purified tropomyosin. A, Gel stained with Coomassie brilliant blue. B, Immunoblotting with the subjects sera. Lane M: molecule weight marker proteins; Lane S: water-soluble shrimp proteins, Lane I: water-insoluble shrimp proteins, T: purified natural tropomyosin

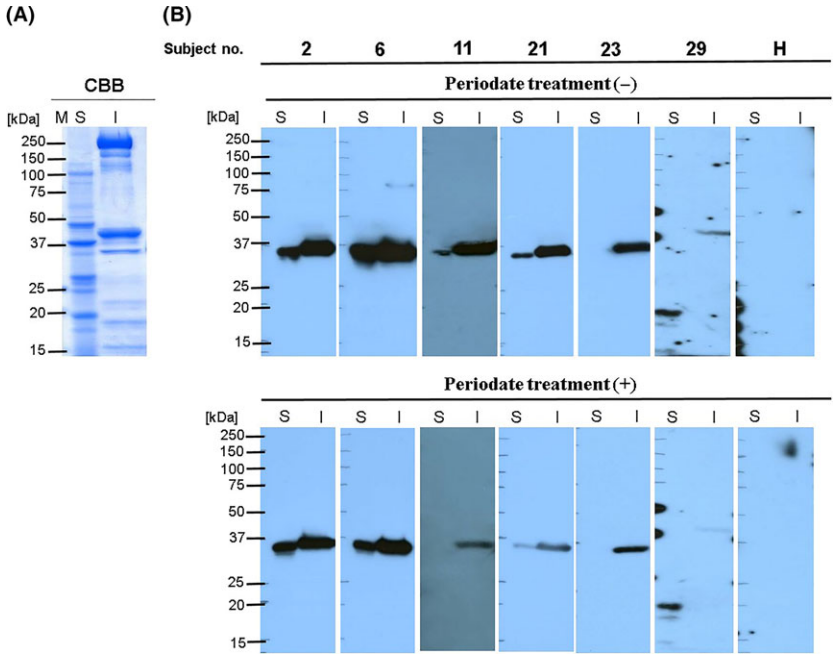

FIGURE 3 IgE immunoblot analysis using shrimp proteins after sodium periodate treatment. A, Gel stained with Coomassie brilliant blue. B, PVDF-blotted. Lane M: molecule weight marker proteins; Lane S: water-soluble shrimp proteins; Lane I: water-insoluble shrimp proteins were incubated with a solution containing periodate (+) or without periodate (−) and were then reacted with patient sera (subjects no: 2, 6, 11, 21, and 23). The subject of 29 was AD: atopic dermatitis and subject H: healthy were used as a negative control

spite of its low sensitivity (37%), the specificity of the tropomyosin-specific IgE test (80%) was much higher than that of the shrimp-specific IgE test (0%). In addition, the findings via immunoblotting were well compatible with the results of tropomyosin-specific IgE tests, supporting that tropomyosin is a highly specific allergen for shrimp allergy. The positive value of the tropomyosin-specific IgE test (1.02 Ua/mL) found in the patient with AD (subject 28) was a non-specific reaction, as no clear band was detected by immunoblotting. Furthermore, the 43-kDa band seen in another patient with AD (subject 29) was considered realistic because of the binding to the carbohydrate moieties irrelevant to tropomyosin, as the binding was diminished after periodate treatment, removing these moieties (Figure 3).

The positivity rate of tropomyosin-specific IgE test in the present study (37%) was far lower than those obtained in the previous studies: Ayuso et al,[21] Gamez et al,[22] and Yang et al[3] reported positivity rates of 57% in the USA, 89% in Spain, and 71.4% in Brazil, respectively. In other studies, involving more than 100 Italian adult patients with shrimp allergy and 38 Singaporean patients with shrimp allergy, only 41% and 15.8%, respectively, were found to be tropomyosin-reactive when investigated by immunoblot analysis and tropomyosin-specific IgE measurements.[23,24] These findings indicate that sensitization to tropomyosin may be dependent on the geographic area, and low sensitization rates are observed in the areas located near the sea, such as Japan, Italy, and Singapore.

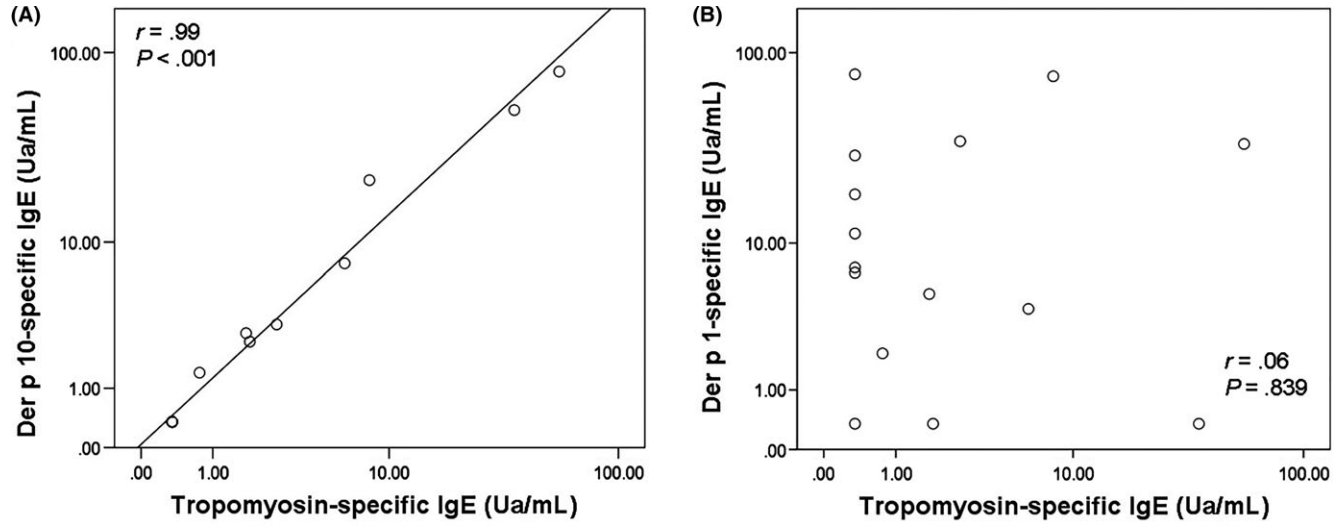

FIGURE 4 Correlations between the level of shrimp tropomyosin-specific IgE and the level of HDMs allergens-specific IgE. A, Correlation of tropomyosin-specific IgE and Der p 10-specific IgE. B, Correlation of tropomyosin-specific IgE and Der p 1-specific IgE

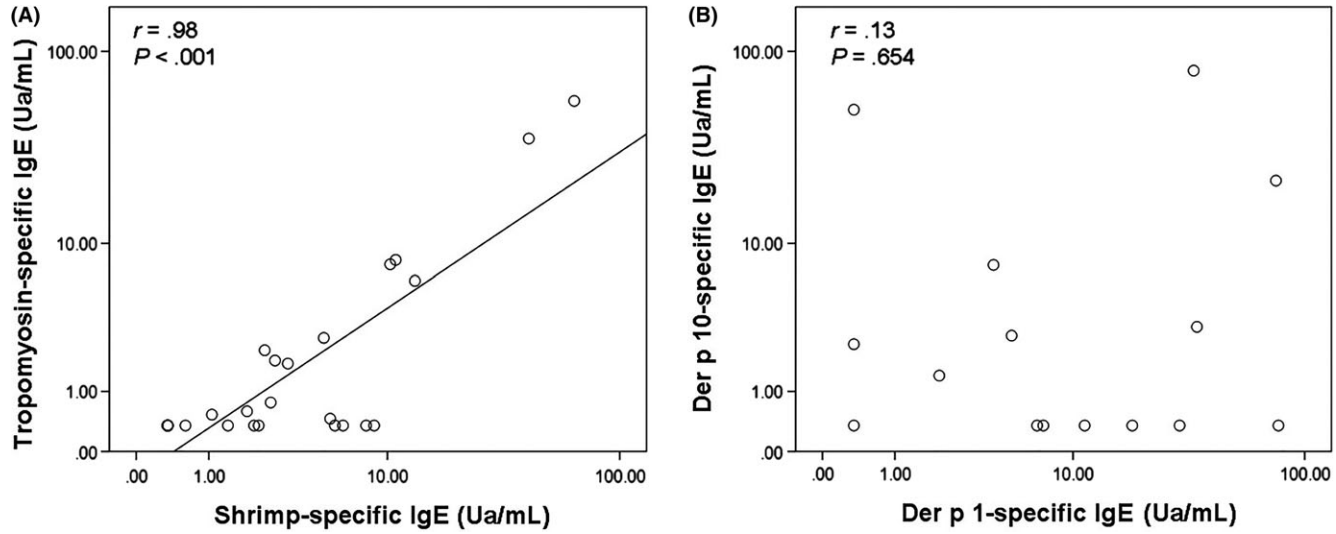

FIGURE 5 Correlations between the level of shrimp-specific IgE and the level of shrimp tropomyosin-specific IgE and between the level of Der p 1-specific IgE and the level of Der p 10-specific IgE. A, Correlation of shrimp-specific IgE and tropomyosin-specific IgE. B, Correlation of Der p 1-specific IgE and Der p 10-specific IgE

In the group I (OAS patients), six of 11 patients showed positive to tropomyosin-specific IgE test (positive rate 54.5%), whereas in the groups II and III (non-OAS patients), only four of 16 patients showed positive to the test (positive rate 25%). From the result, we speculated that the tropomyosin-specific IgE test is associated with OAS symptoms. This finding is consistent with the previous studies; Yang et al[3] and Ayuso et al[21] reported the positivity rates of the tropomyosin-specific IgE test in the patients with OAS as 50% and 100%, respectively. These findings suggest that tropomyosin is a major allergen for OAS caused by shrimp ingestion. In contrast to the patients with OAS (group I), the positivity rate of the tropomyosin-specific IgE test was 23% in the patients with urticaria (group II). The positive results obtained were compatible with the result of immunoblotting, which recognized the tropomyosin band in subjects 21 and 23. The lack of reactivity of subject 22 may be attributed to have low level of tropomyosin-specific IgE test. The shrimp-specific IgE test lacked enough sensitivity to identify patients with urticaria (positivity rate was 61.5%). The lack of reactivity to shrimp allergens in patients with urticaria may be attributed to less sensitivity of ImmnoCAP test and immunoblotting because the skin prick test showed a positive reaction to shrimp allergens in these patients. Previous studies reported similar results; Ayuso et al[21] Kunimoto et al[25] and Adachi et al[26] reported positivity rates of the tropomyosin-specific IgE test in patients with urticaria as 42.1%, 16.6%, and 12.5%, respectively.

Adachi et al[26] reported that a 70-kDa band was detected in 30 of the 31 patients with shrimp allergy (96.7%), suggesting the identification of a new shrimp allergen. These data are different from our results, as we did not find a new allergen of 70 kDa in our experiments. This may be due to the differences in the binding capacity of the secondary antibodies because we used a secondary antibody different from that of Adachi et al.[26]

The reason why the two patients (subjects 23 and 26) showed 34-35 kDa band only in the water-insoluble proteins of shrimp was not clarified in this study, but we speculated that the amount of tropomyosin in the water-soluble fraction is less compared with that in the water-insoluble fraction, because tropomyosin is fundamentally water-insoluble protein. Cross-reactivity between crustaceans and mollusks is also clearly explained by the sensitization to tropomyosin because the nine patients with positive tropomyosin-specific test showed positive IgE against crab and squid. When we analyzed relation between level of tropomyosin-specific IgE and that of Der p 1-specific IgE, a significant correlation was found, although no significant correlation was seen between level of tropomyosin-specific IgE and that of Der p 1-specific IgE (Figure 4). This indicates that shrimp tropomyosin-specific IgE strongly cross-reacts to Der p 10. Here raises a question which is the first, sensitization to shrimp tropomyosin or sensitization to Der p 10. Then, we analyzed relation between level of Der p 1-specific IgE and that of Der p 10-specific IgE, in addition to relation between level of tropomyosin-specific IgE and that of shrimp-specific IgE (Figure 5). As shown in the Figure 5, strong correlation was found between shrimp-specific IgE and tropomyosin-specific IgE, however, no significant correlation was seen between Der p 1-specific IgE and Der p 10-specific IgE, indicating that specific IgE sensitized to shrimp tropomyosin cross-react to HDM tropomyosin.

In the present study, we detected IgE bands in seven of the 27 (26%) patients with shrimp allergy using immunoblotting, whereas the shrimp-specific IgE test was detected in 21 of these 27 patients. In addition, we detected tropomyosin-specific IgE only in 13 of the 27 patients with shrimp allergy, suggesting that there are still undetermined shrimp allergens in other patients and more sensitive tests are required to identify patients with shrimp allergy in Japan. The reason why we found low prevalence of tropomyosin-sensitization in our patients compared with those of the overseas-reports is not clearly elucidated in this study. We speculate that most of the Japanese people would be tolerant to shrimp tropomyosin because they often eat shrimp since childhood, compared with the USA, European, and Brazilian populations who have high sensitization rate to shrimp tropomyosin.

ACKNOWLEDGEMENTS

This research is partly supported by the Practical Research Project for Allergy Diseases and Immunology (Research on Allergic Disease and Immunology) from Japan Agency for Medical Research and Development, AMED and Monbukagakusho Honors Scholarship for Privately-Financed International Students (JASSO).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Onon Tsedendorj conducted this study and wrote the manuscript. Yuko Chinuki and Atsuko Adachi contributed for sample collection. Kiyoe Ueda and Kunie Kohno guided the laboratory experiment and statistical analysis. Eishin Morita supervised this research.

ORCID

Onon Tsedendorj http://orcid.org/0000-0001-9460-3037

REFERENCES

1. Hoffman DR, Day ED, Miller JS. The major heat stable allergen of shrimp. Ann Allergy. 1981;47:17–22.
2. Reese G, Ayuso R, Carle T, Lehrer SB. IgE-binding epitopes of shrimp tropomyosin, the major allergen Pen a 1. Int Arch Allergy Immunol. 1999;118:300–1.
3. Yang AC, Arruda LK, Santos AB, et al. Measurement of IgE antibodies to shrimp tropomyosin is superior to skin prick testing with commercial extract and measurement of IgE to shrimp for predicting clinically relevant allergic reactions after shrimp ingestion. J Allergy Clin Immunol. 2010;125:872–8.
4. Mykles DL, Cotton JL, Taniguchi H, Sano K, Maeda Y. Cloning of tropomyosins from lobster (Homarus americanus) striated muscles: fast and slow isoforms may be generated from the same transcript. J Muscle Res Cell Motil. 1998;19:105–15.
5. Leung PS, Chen YC, Gershwin MR. Identification and molecular characterization of Charybdis feriatus tropomyosin, the major crab allergen. J Allergy Clin Immunol. 1998;102:847–52.
6. Miyazawa H, Fukamachi H, Inagaki Y, et al. Identification of the first major allergen of a squid (Todarodes pacificus). J Allergy Clin Immunol. 1996;98:948–53.
7. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K. Tropomyosin, the major Oyster Crassostrea gigas allergen and its IgE-binding epitopes. J Food Sci. 1998;63:44–7.
8. Ishikawa M, Ishida M, Shimakura K, Nagashima Y, Shiomi K. Purification and IgE-binding epitopes of a major allergen in the gastropod Turbo cornutus. Biosci Biotechnol Biochem. 1998;62:1337–43.
9. Aki T, Kodama T, Fujikawa A, et al. Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, Dermatophagoides farinae. J Allergy Clin Immunol. 1995;96:74–83.
10. Witteman AM, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. Int Arch Allergy Immunol. 1994;105:56–61.
11. Atsurias JA, Gomez-Bayon N, Arilla MC, et al. Molecular characterization of American cockroach tropomyosin (Per a 7), a cross-reactive allergen. J Immunol. 1999;162:4342–8.
12. Ayuso R, Lehrer SB, Reese G. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). Int Arch Allergy Immunol. 2002;127:27–37.
13. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol. 2002;129:38–48.

14. Shafique RH, Inam M, Ismail M, Chaudhary FR. Group 10 allergens (tropomyosins) from house-dust mites may cause covariation of sensitization to allergens from other invertebrates. Allergy Rhinol (Providence). 2012;3:74–90.
15. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy. 1999;29:896–904.
16. Morita E, Kunie K, Matsuo H. Food-dependent exercise-induced anaphylaxis. J Dermatol Sci. 2007;47:109–17.
17. Matsuo H, Dahlström J, Tanaka A, et al. Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. Allergy. 2008;63:233–6.
18. Morita E, Chinuki Y, Takahashi H. Recent advances of in vitro tests for the diagnosis of food-dependent exercise-induced anaphylaxis. J Dermatol Sci. 2013;71:155–9.
19. Leung NY, Wai CY, Shu S, et al. Current immunological and molecular biological perspectives on seafood allergy: a comprehensive review. Clin Rev Allergy Immunol. 2014;46:180–97.
20. Takahashi H, Chinuki Y, Tanaka A, Morita E. Laminin γ-1 and collagen α-1 (VI) chain are galactose-α-1,3-galactose-bound allergens in beef. Allergy. 2014;69:199–207.
21. Ayuso R, Sánchez-Garcia S, Pascal M, et al. Is epitope recognition of shrimp allergens useful to predict clinical reactivity? Clin Exp Allergy. 2012;42:293–304.
22. Gamez C, Sanchez-Garcia S, Ibanez MD, et al. Tropomyosin IgE-positive results are a good predictor of shrimp allergy. Allergy. 2011;66:1375–83.
23. Asero R, Mistrello G, Amato S, et al. Shrimp allergy in italian adults: a multicenter study showing a high prevalence of sensitivity to novel high molecular weight allergens. Int Arch Allergy Immunol. 2012;157:3–10.
24. Thalayasingam M, Gerez IF, Yap GC, et al. Clinical and immunochemical profiles of food challenge proven or anaphylactic shrimp allergy in tropical Singapore. Clin Exp Allergy. 2015;45:687–97.
25. Kunimoto A, Sisino T, Sakai K, et al. Molecular cloning and allergenicity of Pen j 1, a major allergen of kuruma prawn, Penaeus japonicas. Biosci Biotechnol Biochem. 2009;73:840–8.
26. Adachi A, Tanaka A, Chinuki Y, Morita E. Identification of 70 kDa shrimp as a possible new allergen for shrimp allergy. Arerugi. 2013;62:960–7. (in Japanese).

How to cite this article: Tsedendorj O, Chinuki Y, Ueda K, Kohno K, Adachi A, Morita E. Tropomyosin is a minor but distinct allergen in patients with shrimp allergies in Japan. *J Cutan Immunol Allergy*. 2018;1:100–108. https://doi.org/10.1002/cia2.12019