

LETTER TO THE EDITOR

Novel allergen from the freshwater clam and the related allergy

Dear Editor,

Incidence of mollusc allergy reportedly varies between 0.15% and 0.4%.^{1,2} Symptoms are heterogeneous among the affected ranging from mild rashes and hives to life-threatening anaphylactic shock. Tropomyosin is frequently the culprit panallergen in those cross-reactivity studies of molluscs and crustaceans.³ When isolated allergy to molluscs or a single species occurs, however, tropomyosin does not seem to have a relevant role.^{4,5} In these cases, mollusc-specific allergens or even species-specific ones may be involved. *Corbicula japonica*, a freshwater bivalve mollusc, is widely consumed in Japan mainly as an ingredient of “miso” soup. In this report, we present two cases of food allergy to the freshwater clam, in which the sensitization to shrimp tropomyosin was not detected.

Patient 1 was a 69-year-old woman who was referred to our hospital because of erythema of the upper torso and arms accompanied by itching 3 h after the ingestion of freshwater clam soup. No systemic symptom was noted. The patient had experienced the same symptoms 3 years prior after eating clams. There was no episode after eating shrimp, squid and octopus, or foodstuffs devoid of crustaceans and molluscs. No history of fish allergy was registered. Her sister had a history of solar urticaria and her aunt of asthma.

Patient 2 was a 64-year-old man who was rushed to our hospital by ambulance because of face wheal and erythema simultaneously with dyspnea 20 min after ingestion of freshwater clam soup. On arrival, the patient's blood pressure was 116/68 mmHg, heart rate was 125 b.p.m., SpO₂ was 96%, and the erythema extended over the whole body. He suffered from irritable bowel syndrome in 1996 and possible food-associated erythema of the back in the prior year. Unfortunately, the allergens could not be identified.

For both patients there was neither evidence of infection nor use of medication prior to the onset of the disease, and the symptoms were successfully resolved with anti-allergy treatment.

Laboratory investigation showed an elevated concentration of serum immunoglobulin (IgE). Serum-specific IgE antibodies, determined by using ImmunoCAP (Phadia, Uppsala, Sweden), are summarized in Table 1. The results of 2-D electrophoresis and IgE immunoblot is shown in Figure 1(a,c). The main spots were noted for the isoelectric point (IE) 4.5 and 32-kD IgE-binding constituent, which were stained not with the Coomassie Brilliant Blue (CBB) and silver stain liquid, but with the Ruby gel stain liquid (data not shown). When using serum of the healthy control against protein from the shrimp and freshwater clam, very faint bands were barely visible at 37 and 32 kD, respectively (Fig. 1b, lane 3). The impact of boiling on antigenicity is depicted in Figure 1(c) using sera of patients. In comparing raw (lane 1) against boiled (lane 2) freshwater clam, IgE reactivity was dramatically enhanced at 32 kD and attenuated at 37 kD.

Table 1. Radioallergosorbent test with CAP system data (Ua/mL)

Foodstuff	Patient 1	Patient 2
Egg white	<0.35	–
Milk	<0.35	–
Wheat	<0.35	–
Gluten	<0.35	<0.35
Soybean	<0.35	<0.35
Shrimp	<0.35	<0.35
Shrimp tropomyosin	<0.35	–
Squid	<0.35	<0.35
Octopus	–	<0.35
Crab	<0.35	<0.35
Asari (short necked clam)	1.65	1.98
Scallop	1.72	3.28
Total immunoglobulin E	478.9 IU/L	880.8 IU/L

Herein, we report two cases of freshwater clam allergy. Both patients had a clear history of taking freshwater clam, but developed different clinical manifestations. We speculate that the difference may be due to the variations in their degree of sensitization, the dose of exposure to the offending food, and/or the sensitivity of receptors in their various tissues to the specific mediators.⁶

Tropomyosin is known to be the most frequent causative stuff in the seafood allergy and the resultant cross-reactivity is invariably found.⁷ Indeed, the allergens in oyster (*Cra g 1*), abalone (*Hal m 1*) and squid (*Tod p 1*) have been identified as tropomyosin.⁸ However, in our study, the serological and clinical cross-reactivity was not observed between freshwater clam and shrimp. The patients tolerated all crustaceans and cephalopod molluscs, and the sensitization to shrimp was not detected by immunoblotting assay as well as ImmunoCAP. Using 2-D gel electrophoresis, we found that the prominent allergen in the extract of freshwater clam corresponded to a protein with a molecular weight (MW) of approximately 32 kD and an IE of 4.5, different from those of the shrimp tropomyosin (MW 38 kD, IE 5.9). The tropomyosins of crustacean species, in fact, share only 56–68% amino acid sequence identities with those of molluscan shellfish species.⁸ Sometimes, just the minor differences in the structures of tropomyosin between various molluscan and crustacean shellfish species could account for the noted differences in the immunoreactive features. Alternatively, there was evidence for the existence of both common (thus, cross-reactive) and species-specific epitopes (European Food Safety Authority, EFSA, 2006). The diversity in epitopes may likely explain the lack of uniform allergic cross-reactivity that is observed clinically. A third possibility is that unique allergens, other than the tropomyosin, are involved in some species, such as the myosin heavy chain, hemocyanin, arginine kinase and amylase.⁸ Consistently, Leung *et al.*⁹ identified a

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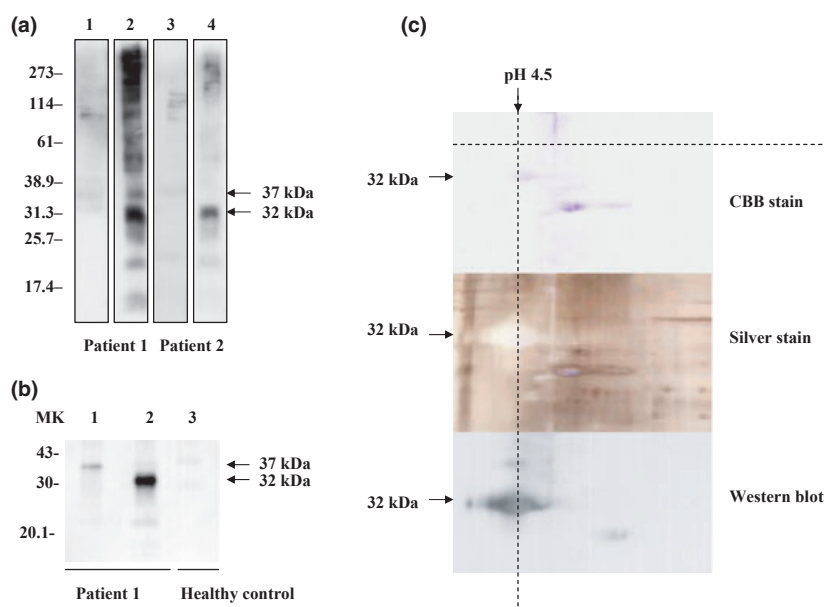


Figure 1. Analyses of shrimp and clam extracts by SDS-PAGE and IgE immunoblotting. (a) Lanes in odd and even number denotes boiled shrimp and boiled freshwater clam, respectively; lane 1 and 2, patient 1; lane 3 and 4, patient 2. (b) Lane 1 indicates raw freshwater clam, lane 2 and 3 indicate boiled freshwater clam. (c) Two dimensional gel electrophoresis of the fresh water clam extracts. Gel was stained by the CBB stain liquid, or silver stain liquid, or analyzed by IgE immunoblotting. The main spot indicates a 32 kD IgE-binding protein with an IE of 4.5, which was not stained by the CBB stain liquid and silver stain liquid.

major allergen in the crab *Charybdis feriatus*, which was similar to the shrimp tropomyosin but with a different MW. Moreover, the main allergen of King Broderip clam was not tropomyosin as well.³ Whether these specific reactions are different epitope-dependent IgE responses or merely the reflection of the first shellfish exposure is currently unknown. These presently unanswered issues hence warrant more clinical studies to better define the frequency of cross-reactions and identify the underlying allergens. Nevertheless, all the existing lines of evidence suggested that there is a specific antigen from the molluscan shellfish.

We happened to observe an interesting phenomenon while processing the gel stain. The main antigen was not chromatic by both the CBB and silver stain liquid, however, the band was clearly visible by the Ruby gel stain. On the other hand, our data implied the configuration change of the specific antigen after boiling. The main band of the raw and boiled extracts was respectively detected at an MW of 37 and 32 kD, with the former similar to the shrimp tropomyosin.⁴ Although the antigen protein was not thermally stable, the antigen specificity was maintained. The 32-kD allergens may play an important role, as only the boiled freshwater clam was eaten, in the ensuing anaphylactic symptoms. Concurrently, our study converged on the structure of the specific allergen.

In short, our results indicate that protein(s) involved in the mollusc allergy seem to be a specific and heat unstable allergen. Sensitization to molluscan shellfish was supported by both *in vivo* and *in vitro* studies. The mechanisms remain unknown and further studies are necessary. We speculated that the cross-reaction might be multifactorial in origin: the existence of the tropomyosin allergy, the homology in the amino acid sequences of the tropomyosins and possible involvement of other specific allergen(s).

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