

LETTER



WILEY

Tropomyosin is no accurate marker allergen for diagnosis of shrimp allergy in Central Europe

To the Editor,

Crustacea shrimp is a frequent cause of food allergy with symptoms ranging from mild oral reactions to severe anaphylaxis. Shrimp contains the single major allergen tropomyosin (TM), which is also present in several invertebrates, for example, house dust mites (HDM). In fact, shrimp allergy has initially been considered to result from IgE-cross-reactivity following sensitization to Der p 10, the TM from *Dermatophagoides pteronissimus*. Because TM-specific IgE was found to be a good predictor of shrimp allergy in HDM-allergic individuals,¹ singleplex assays with shrimp TM found their way into serological routine diagnosis. However, evidence accumulated that additional allergens cause shrimp allergy and cross-reactivity with HDM, such as arginine kinase (AK), myosin light chain (MLC), sarcoplasmic calcium-binding protein (SCBP), hemocyanin (HC), triosephosphatase-isomerase (TPI), and troponin C (TC).² Here, samples from 79 individuals who experienced allergic reactions to shrimps and displayed shrimp-specific IgE in ImmunoCAP (Table S1) were tested after approval by the ethics committee of the Medical University of Vienna (EK 1344/2018) and informed consent for IgE reactivity to recombinant allergens from *Penaeus monodon* and *Crangon crangon* in the newly developed Allergy Explorer-ALEX 2[®] and by immunoblotting to proteins extracted from *Litopenaeus vannamei*, a species frequently consumed in Austria, for example, as “cocktail shrimps” (for details see Appendix S1). The macroarray revealed that 42% of the patients displayed IgE to Pen m 1 (TM, 19% exclusively), 20% to Pen m 2 (AK, 3.7% exclusively), 10% to Pen m 3 (MLC, 2.5% exclusively), and 11% to Cra c 6 (TC), and matched IgE-profiles determined by immunoblotting (Figure 1 and Table S2). Thus, TM represented no major allergen in this cohort. Notably, 30% of the patients recognized Pen m 4 and 16% showed exclusive IgE reactivity to this SCBP with confirmed clinical relevance.³ Therefore, we consider Pen m 4

as important as Pen m 1 for the diagnosis of shrimp allergy in Central Europe. This conclusion is in line with previous studies.³⁻⁵

In accordance with a strong association of shrimp and HDM allergy found by others,⁶ 75% of our patients were sensitized to HDM among who 54% reacted with Pen m 1, 27% with Pen m 2, 17% with Pen m 4, 14% with Cra c 6, and 10% with Pen m 3 (Table S2). Thus, Pen m 1 was the major allergen but no sole diagnostic marker of shrimp allergy as previously reported for Italian HDM-allergic patients.⁶ We found no correlation of IgE levels specific for extracts from shrimp and HDM ($R = .238, p = .072$) and for HDM and Pen m 1 ($R = .149, p = .216$). Nevertheless, IgE levels for Pen m 1 and Der p 10 ($R = .985, p < .001$) and Pen m 2 and Der p 20 ($R = .771, p < .001$) correlated significantly. To confirm that additional allergens are required for an accurate serological diagnosis, we calculated the diagnostic sensitivity of Pen m 1 in combination with the other allergens (Table 1). All recombinant allergens together achieved a sensitivity of 68%. We expect that the inclusion of hemocyanin and ubiquitin might enhance this value as 10% and 9% of the patients recognized the corresponding proteins of 70 and 5–7 kDa, respectively, in the immunoblot (Figure 1). Hemocyanin was proposed to be relevant in the cross-reactivity of shrimps and mites and is mainly contained in the cephalothorax, but may be found in the abdominal muscle. As we employed shrimps without cephalothorax for extract preparation, our results underestimate the frequency of hemocyanin recognition.

The molecular diagnosis approaches combined here further confirm how variate the IgE recognition of shrimp allergens can be, independently of whether patients are sensitized to HDM or not. This study shall raise awareness that TM is no accurate sole marker allergen for the serological diagnosis of shrimp allergy in HDM-sensitized patients.

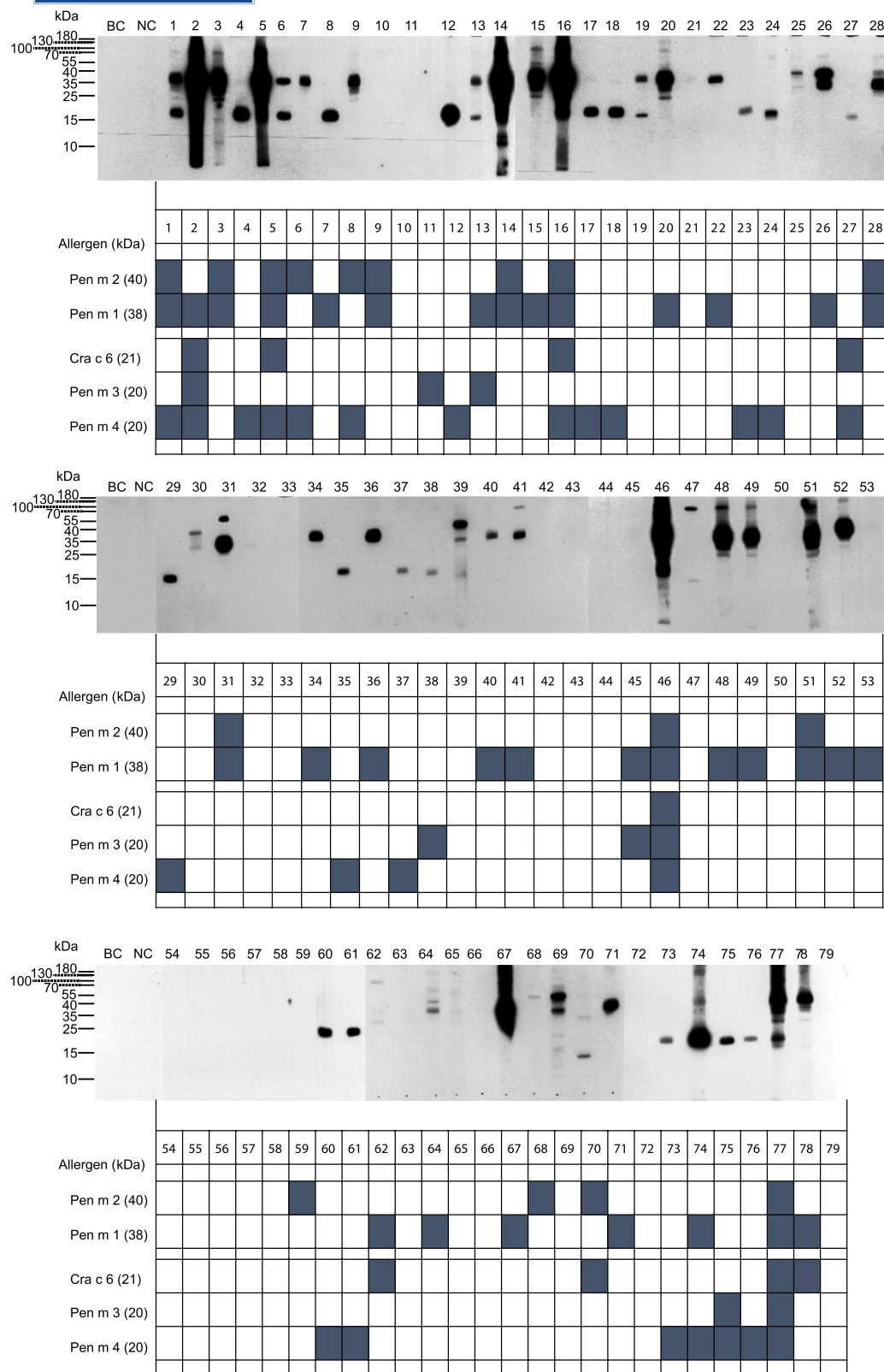


FIGURE 1 IgE-profiling of 79 shrimp-allergic individuals. IgE reactivity to *Litopenaeus vannamei* extract in immunoblot is shown in the upper panel. IgE reactivity to chip-spotted allergens is represented as dark boxes in the lower panel; BC, buffer control, NC, non-allergic control; kDa, kilo Dalton; detailed methodological information is provided in the online supplement

TABLE 1 IgE recognition of recombinant shrimp allergens of 59 HDM-sensitized patients (%)

Pen m 1	54.2
Pen m 1 + Cra c 6	55.9
Pen m 1 + Pen m 3	57.6
Pen m 1 + Pen m 4	59.3
Pen m 1 + Pen m 3 + Pen m 4	59.3
Pen m 1 + Pen m 3 + Cra c 6	59.3
Pen m 1 + Pen m 4 + Cra c 6	61.0
Pen m 1 + Pen m 2	62.7
Pen m 1 + Pen m 2 + Cra c 6	62.7
Pen m 1 + Pen m 2 + Pen m 4	64.4
Pen m 1 + Pen m 2 + Pen m 4 + Cra c 6	64.4
Pen m 1 + Pen m 3 + Pen m 4 + Cra c 6	64.4
Pen m 1 + Pen m 2 + Pen m 3	66.1
Pen m 1 + Pen m 2 + Pen m 3 + Cra c 6	66.1
Pen m 1 + Pen m 2 + Pen m 3 + Pen m 4	67.8
Pen m 1 + Pen m 2 + Pen m 3 + Pen m 4 + Cra c 6	67.8

FUNDING INFORMATION

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

ONLINE SUPPLEMENTARY MATERIAL

**Tropomyosin is no accurate marker allergen for diagnosis of shrimp allergy in
Central Europe**

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Study cohort

Serum samples were collected for routine allergy diagnosis from individuals presenting with shrimp-induced allergic reactions at the Allergy Outpatient Clinic Reumannplatz in Vienna and at the Department of Dermatology and Venereology, Medical University of Graz in Austria. Allergen-specific and total IgE levels were measured using ImmunoCAP 250 (Thermo Fisher Scientific, Waltham, USA). Allergen-specific IgE values $>0.35 \text{ kU}_A/\text{l}$ to the shrimp mix containing *Pandalus borealis*, *Penaeus monodon*, *Metapenaeopsis barbata* and *Metapenaeus joyneri* and to *Dermatophagoides pteronyssinus* were considered positive. Patients' histories of their symptoms after consumption of shrimp were recorded and graduated into local and systemic allergic symptoms. The following symptoms were considered as local reactions: itchy mouth, scratchy throat, swelling of the lips, mouth, tongue, and throat. Systemic allergic reactions comprised generalized skin symptoms (urticaria, angioedema), dizziness, dyspnea, drop of blood pressure, and loss of consciousness. Experiments were performed according to the Declaration of Helsinki principles after approval by the ethics committee of the Medical University of Vienna (EK 1344/2018).

Table S1. Demographics of shrimp-allergic patients (n=79)

Characteristics	
Sex (female/male), n (%)	34/45 (43/57)
Age, median (range)	34 (14-79) years
Local/systemic reactions to shrimp, n (%)	22/57 (28/72)
Total serum IgE, median (25-75% percentile)	221 (108-450) kU/l
shrimp-specific IgE, median (25-75% percentile)	2.6 (1.15-6.1) kU _A /L

Allergy Explorer-ALEX2®

The macroarray Allergy Explorer-ALEX2® (MacroArrayDx, Vienna, Austria) consists of a nitrocellulose membrane onto which nanoparticles activated with different allergens, allergen extracts and other components are spotted. Sera from shrimp-allergic and non-atopic subjects were diluted 1:5 in a diluent supplemented with an inhibitor of cross-reactive carbohydrate determinants (CCD) and incubated on the chips for 2 hours at RT. After washing, an alkaline phosphatase-labeled anti-human IgE antibody was added and incubated for 30 min at RT. Enzyme substrate was added and the intensity of the color reaction for each allergen spot was measured by a couple-charged device camera. The dedicated software digitalizes the images. Calibration is done *via* heterologous calibration against an IgE reference curve. Specific IgE values greater than 0.3 kU_A/l were considered positive.

53 **Table S2.** Sensitization to shrimp and HDM allergens in ImmunoCAP and Allergy
 54 Explorer-ALEX2®

No.	Shrimp CAP	Pen m 1	Pen m 2	Pen m 3	Pen m 4	Cra c 6	HDM CAP	Der p 10	Der p 20
1	4.3	2.3	21.8	<0.3	1.94	<0.3	6.4	1.9	20.2
2	86.8	31.3	<0.3	0.5	10.4	2.5	34.2	30.9	<0.3
3	5.9	5.8	7.2	<0.3	<0.3	<0.3	5.3	6.6	5.8
4	2.3	<0.3	<0.3	<0.3	9.1	<0.3	2.6	<0.3	<0.3
5	34.2	32.5	6.8	<0.3	3.6	4.0	12.6	28.5	8.4
6	6.9	<0.3	14.6	<0.3	1.2	<0.3	0.6	<0.3	10.5
7	0.9	3.4	<0.3	<0.3	<0.3	<0.3	<0.35	2.1	<0.3
8	4.0	<0.3	7.2	<0.3	8.4	<0.3	6.8	<0.3	9.8
9	1.6	3.1	10.7	<0.3	<0.3	<0.3	1.0	5.3	2.3
10	3.9	<0.3	<0.3	<0.3	<0.3	<0.3	4.8	<0.3	<0.3
11	2.5	<0.3	<0.3	1.4	<0.3	<0.3	57.2	<0.3	<0.3
12	9.3	<0.3	<0.3	<0.3	23.8	<0.3	<0.35	<0.3	<0.3
13	1.1	1.6	<0.3	1.6	<0.3	<0.3	11.1	0.7	<0.3
14	100	29.9	10.9	<0.3	<0.3	<0.3	20.2	29.9	20.7
15	3.4	5.5	<0.3	<0.3	<0.3	<0.3	1.3	4.8	<0.3
16	58.7	31.7	10.9	<0.3	7.8	0.8	n.t.	30.2	4.1
17	2.9	<0.3	<0.3	<0.3	9.1	<0.3	<0.35	<0.3	<0.3
18	2.5	<0.3	<0.3	<0.3	7.0	<0.3	<0.35	<0.3	<0.3
19	0.4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.35	<0.3	<0.3
20	6.3	9.6	<0.3	<0.3	<0.3	<0.3	4.4	17.3	<0.3
21	0.5	<0.3	<0.3	<0.3	<0.3	<0.3	2.6	<0.3	<0.3
22	0.6	1.4	<0.3	<0.3	<0.3	<0.3	<0.35	1.2	<0.3
23	0.9	<0.3	<0.3	<0.3	4.6	<0.3	<0.35	<0.3	<0.3
24	5.8	<0.3	<0.3	<0.3	18.4	<0.3	n.t.	<0.3	<0.3
25	2.7	<0.3	<0.3	<0.3	<0.3	<0.3	2.1	<0.3	<0.3
26	0.7	2.9	<0.3	<0.3	<0.3	<0.3	<0.35	1.7	<0.3
27	2.0	<0.3	<0.3	<0.3	6.1	1.5	<0.35	<0.3	<0.3
28	4.5	7.1	0.6	<0.3	<0.3	<0.3	20.5	6.6	1.8

29	3.7	<0.3	<0.3	<0.3	10.6	<0.3	<0.35	<0.3	<0.3
30	1.9	<0.3	<0.3	<0.3	<0.3	<0.3	1.6	<0.3	<0.3
31	17.3	22.9	1.1	<0.3	<0.3	<0.3	2.8	19.2	8.2
32	1.4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.35	<0.3	<0.3
33	0.9	<0.3	<0.3	<0.3	<0.3	<0.3	1.1	<0.3	<0.3
34	1.0	4.6	<0.3	<0.3	<0.3	<0.3	63.2	2.3	<0.3
35	1.4	<0.3	<0.3	<0.3	6.7	<0.3	<0.35	<0.3	<0.3
36	2.6	7.4	<0.3	<0.3	<0.3	<0.3	0.8	6.9	<0.3
37	0.7	<0.3	<0.3	<0.3	4.1	<0.3	<0.35	<0.3	<0.3
38	0.5	<0.3	0.2	1.4	<0.3	<0.3	5.5	<0.3	<0.3
39	2.1	<0.3	<0.3	<0.3	<0.3	<0.3	n.t.	<0.3	<0.3
40	0.9	3.1	<0.3	<0.3	<0.3	<0.3	1.6	4.4	<0.3
41	3.1	6.3	<0.3	<0.3	0.2	<0.3	2.3	4.5	<0.3
42	4.1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.35	<0.3	<0.3
43	0.9	<0.3	<0.3	<0.3	<0.3	<0.3	3.7	<0.3	<0.3
44	0.6	<0.3	<0.3	<0.3	<0.3	<0.3	3.9	<0.3	<0.3
45	1.6	0.4	<0.3	1.6	<0.3	<0.3	n.t.	<0.3	<0.3
46	54.6	16.8	24.4	19.1	11.2	8.3	5.0	11.4	13.6
47	1.2	<0.3	<0.3	<0.3	<0.3	<0.3	6.7	<0.3	<0.3
48	24.7	26.54	<0.3	<0.3	<0.3	<0.3	8.8	27.2	<0.3
49	23.9	27.0	0.29	<0.3	<0.3	<0.3	14.7	25.1	0.4
50	0.9	<0.3	<0.3	<0.3	<0.3	<0.3	0.8	<0.3	<0.3
51	11.0	16.9	5.4	<0.3	<0.3	<0.3	5.3	13.2	5.0
52	20.6	27.1	<0.3	<0.3	<0.3	<0.3	5.5	25.0	<0.3
53	1.9	1.8	<0.3	<0.3	<0.3	<0.3	3.1	1.2	<0.3
54	4.0	<0.3	<0.3	<0.3	<0.3	<0.3	9.0	<0.3	<0.3
55	0.5	<0.3	<0.3	<0.3	<0.3	<0.3	0.5	<0.3	<0.3
56	0.7	<0.3	<0.3	<0.3	<0.3	<0.3	0.8	<0.3	<0.3
57	0.7	<0.3	<0.3	<0.3	<0.3	<0.3	n.t.	<0.3	<0.3
58	2.7	<0.3	<0.3	<0.3	<0.3	<0.3	3.4	<0.3	<0.3
59	2.3	<0.3	10.5	<0.3	<0.3	<0.3	33.1	<0.3	6.6
60	5.9	<0.3	<0.3	<0.3	20.4	<0.3	n.t.	<0.3	<0.3

61	5.7	<0.3	<0.3	<0.3	22.0	<0.3	n.t.	<0.3	<0.3
62	3.7	0.3	<0.3	<0.3	<0.3	4.3	1.8	0.5	<0.3
63	48.8	<0.3	<0.3	<0.3	<0.3	<0.3	27.4	<0.3	<0.3
64	9.7	1.3	<0.3	<0.3	<0.3	<0.3	40.9	<0.3	<0.3
65	4.4	<0.3	<0.3	<0.3	<0.3	<0.3	19.7	<0.3	<0.3
66	23.4	<0.3	<0.3	<0.3	<0.3	<0.3	2.8	<0.3	<0.3
67	0.5	23.5	<0.3	<0.3	<0.3	<0.3	<0.35	17.3	<0.3
68	1.2	<0.3	0.7	<0.3	<0.3	<0.3	0.5	<0.3	1.1
69	1.5	<0.3	<0.3	<0.3	<0.3	<0.3	0.4	<0.3	<0.3
70	1.6	<0.3	4.3	<0.3	<0.3	7.5	2.3	<0.3	21.6
71	1.	9.6	<0.3	<0.3	<0.3	<0.3	15.1	8.9	<0.3
72	1.3	<0.3	<0.3	<0.3	<0.3	<0.3	1.2	<0.3	<0.3
73	20.7	<0.3	<0.3	<0.3	26.7	<0.3	<0.35	<0.3	<0.3
74	5.5	0.9	<0.3	<0.3	33.2	<0.3	53.6	0.6	<0.3
75	57.1	<0.3	<0.3	3.1	9.1	<0.3	<0.35	<0.3	<0.3
76	1.4	<0.3	<0.3	<0.3	6.5	<0.3	<0.35	<0.3	<0.3
77	21.3	10.1	13.1	9.4	6.1	4.2	2.7	7.4	6.4
78	8.7	9.5	<0.3	<0.3	<0.3	3.8	6.7	8.3	<0.3
79	0.5	<0.3	<0.3	<0.3	<0.3	<0.3	0.7	<0.3	<0.3

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57 **Immunoblotting studies**

58 For IgE-profiling to natural shrimp allergens we employed *Litopenaeus vannamei* (*Lit v*,
59 whiteleg shrimp) because this species is frequently purchased and consumed in Austria
60 and was therefore expected to be the cause of most allergic reactions in our study
61 cohort. Furthermore, allergens described in *Pen m* have also been identified in *Lit v* (1-
62 3). Four frozen, blanched, and beheaded (absent cephalothorax) whiteleg shrimps with
63 a total weight of approximately 58 g were deveined and their tail shells were removed.
64 Four tablets of EDTA-free cOmplete™ protease inhibitor cocktail (F. Hoffmann-La

Roche AG, Basel, Switzerland) were dissolved in 200 ml of PBS at 4 °C. Shrimps were added and homogenized in an electrical blender. The suspension was centrifuged at 15.000 x g for 20 min at 4 °C. The supernatant was collected and dialyzed against PBS at 4 °C using a 3.5 kDa cutoff membrane. The protein concentration was determined by BCA™ Protein Assay Kit (Thermo Fisher Pierce, Rockford, IL, USA). The protein extract was aliquoted and stored at -20 °C until further use.

The *Lit v* protein extract (330 µg) was separated after cooking at 95°C for 3 minutes by 15% SDS-PAGE under reducing conditions and electroblotted onto a nitrocellulose membrane (Cytiva, Malborough, MA, USA) for 60 minutes at 4 °C. Nitrocellulose membrane strips (0.5 cm) were cut and saturated with gold buffer, which is PBS supplemented with 0.5% Tween 20, 0.5% BSA, and 0.05% NaN₃ for 30 min at RT. Then strips were incubated with the individual sera from shrimp-allergic and non-atopic subjects (diluted 1:4 in gold buffer) overnight at 4 °C. After washing, bound IgE was detected with a ¹²⁵I-labelled anti-human IgE antibody (Demeditec Diagnostics, Kiel, Germany, diluted 1:10 in gold buffer) incubated overnight at 4 °C. After washing, strips were left to dry at room temperature and exposed to X-ray films at -80 °C for 3 to 5 days.

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