DR SANDIP D KAMATH (Orcid ID : 0000-0002-5956-8552)

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IgE reactivity to shrimp allergens in infants and their cross-reactivity to house dust mite.

AUTHORS:

*Sandip D. Kamath^{1,2,3,5} #, Elecia B. Johnston^{1,2,3} #, Swati Iyer^{1,2,3}, Patrick M. Schaeffer^{1,3,4}, Jennifer Koplin^{5,6}, Katrina Allen^{5,6}, *Andreas L. Lopata^{1,2,3,5}

Affiliations:

¹ Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia;

² Molecular Allergy Research Laboratory, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia;

³ Centre for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Townsville, Australia;

⁴ Supramolecular & Synthetic Biology Group, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia;

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⁵ Centre for Food Allergy and Research, Murdoch Childrens Research Institute, Melbourne, Australia;

⁶ University of Melbourne, Melbourne, Victoria, Australia;

* Joint corresponding authors:

Equal First Authors

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Corresponding authors-

Dr. Sandip D. Kamath and Prof. Andreas L. Lopata

Australian Institute of Tropical Health and Medicine,

James Cook University, DB47 (Pharmacy and Medical Research), 1 James Cook Drive,

Douglas, Townsville, QLD 4811, Australia

sandip.kamath@jcu.edu.au

(W) +61(07)47814846

To the Editor,

The rise in shellfish allergy incidence, accompanying an increase in consumption and exposure to shellfish allergens, has become a serious problem worldwide. Precise diagnosis of shellfish allergy poses a challenge due to the diversity of consumed species, and immunological cross-reactivity to similar invertebrate pan-allergens. Moreover, variable concentrations of major and minor allergens in diagnostic preparations often result in false-negative results. This problem is further exacerbated in pediatric patients with low IgE titers and in many instances, an inaccurate parent-reported history of clinical reactions to shrimp. Studies investigating pediatric shrimp allergy have largely focused on children between 3 and 15 years of age, resulting in a paucity of data on sensitization to shrimp allergens among infants. Until recently, it was believed that dietary introduction to potentially allergenic foods, including shellfish, should be restricted until an infant is at least 36 months of age (1). However, there is growing evidence that early exposure to these foods is beneficial,

increasing the capacity for tolerance (1, 2). The routes of exposure to these allergens are varied. In addition to oral ingestion, sensitivity to food allergens by inhalation or skin contact have also been reported (3). Furthermore, infants are often exposed to invertebrate allergens from insects and house dust mites (HDM), before or concurrent with consumption of shrimp-containing food in their diet. The major pan-allergen Tropomyosin (TM) is believed to be a good biomarker for shrimp allergy, with a positive predictive value of 0.72 (4). Furthermore, seafood – mite cross-reactivity has been previously evaluated, and the main implicated allergen is tropomyosin (5). The purpose of this study was to analyze IgE reactivity against the shrimp allergen tropomyosin in shrimp-sensitized infants, and assess whether other known allergens elicit similar reactivity. We also sought to compare cross-reactivity of these shrimp allergens in HDM-allergic infants.

A total of 66 infants with an average age of 13.9 months, who were a part of the HealthNuts cohort conducted in Melbourne, Australia (6), were selected for this study. The infants were subdivided into three groups – A) Shrimp sensitized (n=18), B) HDM-allergic (n=18) and C) Parent reported reactions to shrimp but SPT negative (n=12) and D) atopic infant controls with no sensitization to shrimp or HDM (n=18). Specific IgE to the major shrimp allergen tropomyosin was confirmed using a recently developed sensitive immuno-PCR assay, which detects IgE in the mobile phase compared to the immobilized matrix-based analysis of immunoblots (Fig. S1) (7). 27% of the 18 shrimp-sensitized infants elicited IgE binding to shrimp tropomyosin by immuno-PCR (Fig. 1A, Table 1). Binding of IgE to shrimp allergens was then further analyzed by immunoblot against shrimp protein extract. 28% of the cohort elicited IgE binding to shrimp tropomyosin (Fig. 1A, D Table 1, 2). In contrast, only 5% of HDM-allergic infants reacted to shrimp tropomyosin by immuno-PCR (Fig. 1B, Table 1), and 11% by immunoblot (Fig. 1B, E, Table 1). No IgE binding was observed on immunoblot among parent-reported shrimp reactive group, except one positive infant by immuno-PCR (Fig. 1C). This result is in concurrence with the SPT outcomes of no positive diagnosis of shrimp reactivity and confirms the negative predictive value of immunoblotting in this study. This group of infants may have adverse reactions to shrimps that are non-IgE mediated, for example, to metabisulphites, which is commonly used as a preservative for shrimps (8). No significant IgE binding was observed in atopic controls (Fig. 1D).

85 Interestingly, we also observed IgE binding to shrimp proteins other than tropomyosin. To
86 confirm their identity, the positive bands were excised and analyzed using mass spectrometry
87 (Table 2). We observed that 22% of the 18 shrimp-sensitized infants displayed reactivity to
88 sarcoplasmic calcium binding protein (SCP) (19 kDa), 33% to hemocyanin (HM) A and B
89 (73 and 64 kDa, respectively), and 11% to enolase (46 kDa) (Fig. 1A, E, Table 1, 2).
90 However, in the HDM-allergic cohort, 61% of the 18 infants displayed reactivity to
91 hemocyanin and 44% to enolase, while no IgE binding was observed to SCP (Fig. 1B, E,
92 Table 1, 2).

93 Sarcoplasmic calcium binding protein has been previously shown to have a high IgE binding
94 prevalence in shrimp-allergic children, and is a good predictor of clinical reactivity to shrimp
95 in conjunction to tropomyosin. However, the lack of IgE binding to sarcoplasmic calcium
96 binding protein in HDM-allergic infants confirms that it is a specific shrimp allergen and
97 might not play a role in cross-sensitization between shrimp and HDM. Hemocyanin, a
98 metalloprotein involved in oxygen transport, has previously been characterized as shrimp
99 allergen (9), and detailed IgE binding analysis has been performed (10). It has also been
100 identified as a group 3 cockroach allergen (Per a 3 and Bla g 3). It is thus plausible that these
101 infants are sensitized to cockroach or other invertebrate inhalant allergens, resulting in IgE
102 cross-reactivity to shrimp hemocyanin. Interestingly, IgE reactivity to these identified
103 allergens was demonstrated in six (30%) shrimp allergic infants, which were negative to
104 tropomyosin by immunoblot as well as immuno-PCR.

105 One additional allergen, enolase, has not been identified as an HDM allergen, while IgE
106 recognition has been demonstrated to cockroach and banana shrimp enolase. Therefore, this
107 protein may function as a cross-reactive allergen between mite- and insect-sensitized
108 individuals to shrimp allergens.

109 Cross-reactivity to shrimp allergens in HDM-allergic patients has been previously
110 demonstrated (9). In our study, we assessed IgE binding by immunoblotting using
111 densitometric analysis, by setting an arbitrary cut-off for positive IgE bands. However, a
112 caveat of this study is that though we have identified that more than 60% of HDM-allergic
113 infants were positive for hemocyanin, and 44% for enolase, cross-reactivity to shrimp
114 allergens can only be confirmed by analyzing IgE reactivity to purified or recombinant
115 hemocyanin and enolase. Another limitation is that hemocyanin and enolase are not

registered crustacean allergens in the IUIS allergen nomenclature, indicating that further investigations are required to confirm allergenicity.

Recent research indicates that the presence of oligosaccharide moieties on allergenic proteins, as well as their glycosylation patterns, may contribute to allergenicity (11). Interestingly, shrimp hemocyanin is glycosylated (9); this modification could cause changes in the IgE reactivity of this glycoprotein (12). The IgE cross-reactivity of HDM-allergic patients to shrimp hemocyanin could be explained by two possibilities; firstly, the HDM-allergic patients may possess IgE against HDM hemocyanin, which may cross-react with shrimp hemocyanin (9) and secondly, that these patients may have IgE antibodies to cross-reactive carbohydrate determinants, which may be responsible for cross-reactivity to the oligosaccharide moieties in shrimp hemocyanin. Detailed molecular analyses are required to determine the role of oligosaccharide moieties on the allergenicity and cross-reactive potential of hemocyanin.

In summary, we have established IgE binding not just to tropomyosin, but also to hemocyanin and enolase in both shrimp-sensitized and HDM-allergic infants. Moreover, we have described cross-reactive IgE binding to shrimp hemocyanin and enolase in HDM-allergic infants, which may have a significant impact on cross-reactivity to shellfish allergens in children undergoing HDM therapy (13). Furthermore, the inclusion of individual shrimp allergens for IgE detection in infants might improve the current diagnostic specificity of allergic sensitization to shrimp, especially in tropomyosin-negative patients. Additionally, knowledge of these allergens will be beneficial for performing longitudinal studies evaluating their potential cross-reactivity to other related food and inhalant allergens. Based on the IgE antibody recognition patterns between different groups, performing a follow-up study on the same or similar cohorts will help evaluate and confirm the potential of these shrimp allergens as early indicators of shrimp allergy. The change in sentiment towards early introduction of a variety of foods antigens (1), including shellfish, to infants necessitates further research on causative allergens, especially in paediatric cohorts at a higher risk of developing allergic disease.

Sandip D. Kamath^{1,2,3,5} M.Sc.,Ph.D., Elecia B. Johnston^{1,2,3} B.Sc. (Hons), Ph.D., Swati Iyer^{1,2,3} M.Sc.,Ph.D,
Patrick M. Schaeffer^{1,3,4} Ph.D, Jennifer Koplin^{5,6} B.Sc. (Hons), Ph.D, Katrina Allen^{5,6} MBBS, FRACP, Ph.D.,
Andreas L. Lopata^{1,2,3,5} M.Sc.,Ph.D

Affiliations:

¹ Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia;

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³ Centre for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Townsville, Australia;

⁴ Supramolecular & Synthetic Biology Group, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Australia;

⁵ Centre for Food Allergy and Research, Murdoch Childrens Research Institute, Melbourne, Australia;

⁶ University of Melbourne, Melbourne, Victoria, Australia;

E-mail: sandip.kamath@jcu.edu.au

References:

1 Reynolds LA, Finlay BB. Early life factors that affect allergy development. *Nat Rev Immunol.* 2017.

2 Chin B, Chan ES, Goldman RD. Early exposure to food and food allergy in children. *Can Fam Physician.* 2014; **60**: 338-9.

3 Ramirez DA, Jr., Bahna SL. Food hypersensitivity by inhalation. *Clinical and molecular allergy : CMA.* 2009; **7**: 4.

4 Gámez C, Sánchez-García S, Ibáñez MD, *et al.* Tropomyosin IgE-positive results are a good predictor of shrimp allergy. *Allergy.* 2011; **66**: 1375-83.

5 Wong L, Huang CH, Lee BW. Shellfish and House Dust Mite Allergies: Is the Link Tropomyosin? *Allergy, asthma & immunology research.* 2016; **8**: 101-6.

6 Osborne NJ, Koplin JJ, Martin PE, *et al.* Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. *J Allergy Clin Immunol.* 2011; **127**: 668-U188.

- 7 Johnston EB, Kamath SD, Lopata AL, Schaeffer PM. Tus-Ter-lock immuno-PCR assays for the sensitive detection of tropomyosin-specific IgE antibodies. *Bioanalysis*. 2014; **6**: 465-76.
- 8 Vitaliti G, Guglielmo F, Giunta L, Pavone P, Falsaperla R. Sodium metabisulphite allergy with multiple food and drug hypersensitivities in a five-year-old child: a case report and literature review. *Allergol Immunopathol (Madr)*. 2015; **43**: 106-8.
- 9 Giuffrida MG, Villalta D, Mistrello G, Amato S, Asero R. Shrimp allergy beyond Tropomyosin in Italy: clinical relevance of Arginine Kinase, Sarcoplasmic calcium binding protein and Hemocyanin. *Eur Ann Allergy Clin Immunol*. 2014; **46**: 172-7.
- 10 Pascal M, Grishina G, Yang AC, *et al*. Molecular Diagnosis of Shrimp Allergy: Efficiency of Several Allergens to Predict Clinical Reactivity. *Journal of Allergy and Clinical Immunology - In practice*. 2015; **3**: 521-29 e10.
- 11 Al-Ghouleh A, Johal R, Sharquie IK, *et al*. The glycosylation pattern of common allergens: the recognition and uptake of Der p 1 by epithelial and dendritic cells is carbohydrate dependent. *PLoS One*. 2012; **7**: e33929.
- 12 van Ree R, Cabanes-Macheteau M, Akkerdaas J, *et al*. Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J Biol Chem*. 2000; **275**: 11451-8.
- 13 van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. *Allergy*. 1996; **51**: 108-13.

Figure 1. IgE reactivity to Black tiger shrimp (*Penaeus monodon*) allergens using immunoblotting (top), and tropomyosin-specific immunoPCR (bottom) for A) shrimp-sensitized infants (n=18); B)

HDM-allergic infants (n=18); C) Infants with parent-reported shrimp allergy but negative to SPT (n=12) and D) Atopic control infants (n=18). The intensity of bands on the immunoblots was quantified by densitometry, and correspond to the numbers listed in Table 1. The dotted line shown on the immuno-PCR bar graph indicates the cut-off for a positive result (<24.5 cycle threshold, Ct). Data represented by mean and SD, n=3; E) Coomassie stained SDS-PAGE of Black tiger shrimp protein extract and location of frequent IgE binding proteins identified by mass spectrometry.

Table 1: Patient demographic and IgE binding data for Group A: Shrimp sensitized infants, and Group B: HDM allergic infants. Positive outcomes highlighted in green, and negative in red. “ND” indicates test Not Done. Negative cut-off values: SPT <1mm, immuno-PCR >24.5 Ct, immunoblot <2 (Immunoblot values are from densitometric analysis, x1000 pixel value).

No.	Age (m)	Sex	SPT Shrimp (mm)	SPT HDM (mm)	Specific IgE to shrimp TM (Ct) (immuno-PCR)	IgE recognition of shrimp allergens on immunoblot (Density)			
						Tropomyosin	Sarcoplasmic calcium binding protein	Hemocyanin	Enolase
Group A – Shrimp sensitized infants									
A1	15.2	F	1	0	24.96	0	0	0	1.17
A2	13.5	M	2	0	25.60	0	0	1.114	10.27
A3	15.8	M	3	7.5	20.51	5.505	39.935	0.989	0
A4	13.8	M	1	0	28.59	0	0	18.147	0.817
A5	14.8	M	5	2	27.63	0	0	31.256	0
A6	12.6	F	3	0	27.21	0	0	2.201	1.045
A7	15.2	M	3.5	0	27.36	1.12	0	0	0
A8	15.8	M	2	0	24.75	18.965	18.226	0.584	0
A9	16	M	2	0	23.23	12.156	9.684	1.547	1.45
A10	13.2	M	1	0	28.23	0	0	0.58	0
A11	11.4	M	3	6.5	21.47	12.085	0	1.399	0.622
A12	14.1	M	5	0	21.29	9.658	41.325	3.649	0.597
A13	13.5	M	1	0	26.91	0	0	1.359	1.048
A14	15.3	M	1	0	27.61	0	0	2.205	1.451
A15	13.1	M	4	0	26.88	0	0	0	0.232
A16	14.7	F	2	0	26.76	0	0	0	0.727
A17	13.4	M	4	0	27.78	0	0	2.437	0.553
A18	13.9	M	2	5	23.60	0.878	0	0.641	2.827
Group B – House Dust Mites allergic infants									
B1	13.1	M	0	3	25.26	0	0	4.222	4.481
B2	14.9	F	0	3	24.69	0	0	2.127	1.58
B3	13.1	M	0	3	27.80	0	0	0.659	0
B4	13.4	F	0	7	27.22	0	0	1.077	1.409
B5	12.8	M	0	9	26.83	0	0	2.025	1.075
B6	14.1	M	0	4	26.99	0	0	3.276	3.708

B7	12.5	F	0	4	27.99	0	0	0	0
B8	14.1	M	0	7.5	22.57	15.366	0	1.951	2.221
B9	12	M	0	11	27.64	2.628	0	2.452	2.101
B10	14.1	M	0	5	28.73	0	0	1.858	1.197
B11	12.5	M	0	5.5	27.64	0	0	4.349	3.686
B12	12.3	F	0	4.5	27.41	0	0	3.841	2.131
B13	12.3	M	0	3	27.11	0	0	12.628	7.066
B14	12.6	M	0	9	27.13	0	0	2.688	1.987
B15	12.2	F	0	5	28.64	0	0	4.161	4.513
B16	14.3	M	0	5	27.54	0	0	1.352	1.646
B17	12.8	F	0	9	27.72	0	0	1.388	1.564
B18	12.2	M	0	5	27.36	0	0	2.566	1.625
Positive test=			>=1	>=1	<24.5	>2	>2	>2	>2

Table 2: Mass spectrometric identification of IgE binding shrimp proteins.

No.	Estimated MW of Band (kDa)	Identified allergens	MW of native protein (kDa)	Mascot score	Percent coverage of total protein sequence	Species (common name)
1	73 kDa	Hemocyanin A chain	75.65 kDa	80.78	1.98%	<i>Astacus leptodactylus</i> (Turkish narrow-clawed crayfish)
2	64 kDa	Hemocyanin B chain	65.25 kDa	141.22	3.71%	<i>Astacus leptodactylus</i> (Turkish narrow-clawed crayfish)
		Peptidase 1	33.20 kDa	35.02	3.11%	<i>Psoroptes ovis</i> (Sheep scab mite)
3	46 kDa	Enolase	47.02 kDa	113.87	4.16%	<i>Homarus gammarus</i> (European lobster)
4	40 kDa	Arginine kinase	40.12 kDa	425.6	19.38%	<i>Penaeus monodon</i> (Black tiger prawn)
		Enolase	47.02 kDa	131.91	6%	<i>Homarus gammarus</i> (European lobster)
		Fructose-bisphosphate aldolase	39.02 kDa	61.05	2.22%	<i>Drosophila melanogaster</i> (Fruit fly)
5	35 kDa	Tropomyosin	32.83 kDa	651.9	31.34%	<i>Penaeus monodon</i> (Black tiger prawn)
		Enolase	47.01 kDa	118.58	4.16%	<i>Homarus gammarus</i> (European lobster)
		Fructose-bisphosphate aldolase	39.02 kDa	46.36	2.22%	<i>Drosophila melanogaster</i> (Fruit fly)
6	27 kDa	Arginine kinase	40.12 kDa	1183.34	51.12%	<i>Penaeus monodon</i> (Black tiger prawn)
		14-3-3 protein zeta	28.08 kDa	393.59	27.53%	<i>Bombyx mori</i> (Silk moth)
		Troponin I	23.48 kDa	188.06	14.43%	<i>Astacus leptodactylus</i> (Turkish narrow-clawed crayfish)
7	19 kDa	Sarcoplasmic calcium-binding protein, beta chain	21.95 kDa	911.95	63.02%	<i>Penaeus sp.</i> (Penaeid shrimp)
		Sarcoplasmic calcium-binding protein, alpha-B and -A chains	21.96 kDa	444.69	35.94%	<i>Penaeus sp.</i> (Penaeid shrimp)
		Troponin I	23.47 kDa	278.96	14.93%	<i>Astacus leptodactylus</i> (Turkish narrow-clawed crayfish)

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Author/s:

Kamath, SD;Johnston, EB;Iyer, S;Schaeffer, PM;Koplin, J;Allen, K;Lopata, AL

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