

New insights into seafood allergy

Andreas L. Lopata^a and Samuel B. Lehrer^b

^aRMIT University, Bundoora West Campus, Melbourne, Victoria, Australia and ^bTulane University, New Orleans, Los Angeles, USA

Correspondence to Andreas L. Lopata, Associate Professor, RMIT University, Bundoora West Campus, Melbourne, VIC 3083, Australia
Tel: +61 3 9925 6627; fax: +61 3 9925 7110;
e-mail: andreas.lopat@rmit.edu.au

Current Opinion in Allergy and Clinical Immunology 2009, 9:000–000

Purpose of review

Seafood plays an important role in human nutrition worldwide, sustained by international trade of a variety of new seafood products. Increased production and consumption have resulted in more frequent reports of adverse reactions, highlighting the need for more specific diagnosis and treatment of seafood allergy. This review discusses recent literature in this field.

Recent findings

The most recent prevalence data from Asia highlight seafood as a significant sensitizer in up to 40% of children and 33% of adults. Furthermore, the demonstration of species-specific sensitization to salt-water and fresh-water prawns and processed prawn extract should improve diagnosis. Studies on humans demonstrated for the first time that biologically active fish allergens can be detected in serum samples as early as 10 min after ingestion. These studies highlight that minute amounts of ingested seafood allergens can quickly trigger allergic symptoms; also, inhaled airborne allergens seem to induce sensitization and reactions. In the past 2 years, over 10 additional seafood allergens have been characterized. Allergen-specific detection assays in food products are available for crustacean tropomyosin; however, many specific mollusk and some fish allergens are not readily identified.

Summary

Although cross-reactivity between crustacean and mollusks as well as mites is demonstrated, the often poor correlation of IgE reactivity and clinical symptoms calls for more detailed investigations. The recent development of hypoallergenic parvalbumin from carp could form the basis for safer vaccination products for treatment of fish allergy. Molecular characterization of more universal marker allergens for the three major seafood groups will improve current component-resolved clinical diagnosis and have a significant impact on the management of allergic patients, on food labeling and on future immunotherapy for seafood allergy.

Keywords

Anisakis, parvalbumin, seafood allergy, shellfish, tropomyosin

Curr Opin Allergy Clin Immunol 9:000–000
© 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

Seafood plays an important role in human nutrition and health. The growing international trade in seafood reflects the popularity and frequency of consumption of a variety of seafood products across many countries. Unfortunately, increased production and consumption of seafood have resulted in more frequent adverse health problems among consumers of seafood. Adverse reactions to seafood can be mediated by the immune system (allergies) as well as by nonimmunological mechanisms. The two invertebrate phyla, crustacean and mollusks, are generally referred to as 'shellfish' in the context of seafood consumption. The group of fish can be subdivided into the bony fish, to which most edible species belong, and the cartilaginous fish (sharks and rays). Most studies of fish allergens have

focused on cod and carp; however, fish consumption, thus exposure (and subsequently allergy), depends much on regional availability.

Epidemiology and prevalence

The prevalence of seafood allergy is usually higher when the consumption plays a greater part in the diet of the observed community. It is generally considered that crustaceans and fish are among the four food groups most commonly provoking severe food anaphylaxis. The consumption of seafood has increased worldwide, and therefore, the imports of seafood from other countries often surpass the own productions. In the USA, the third largest global consumer of fish and shellfish behind Japan and China [1], over 80% of seafood is imported. A recent

analysis of food-related reactions in emergency departments in the USA established that shellfish was the most frequently implicated food in persons aged 6 years or older [2]. It is estimated that about 30 000 food-induced anaphylactic events are seen annually in the USA alone, of which about 200 or more are fatal. A recent study by Vierk *et al.* [3•] provided population-based prevalence data among US adults from a Food Safety Survey among over 4400 individuals. The prevalence of shellfish allergy was found to be 1.9 and 1.3% among respondents with self-reported food allergy and self-reported doctor-diagnosed food allergy, respectively. Overall, there was no difference in the prevalence of food allergy among age or race/ethnic groups. However, significantly more black than white respondent's reported a fish, shellfish or crustacean food allergy. A similar observation was made by Sicherer *et al.* [4] in a telephone survey among 14 948 individuals. The reasons for these unexpected observations are unknown and require further studies among this demographic group.

Seafood allergy is common not only in western but also in Asian countries where allergic reactions to seafood and particularly shellfish are significant among children and adults [5]. A study from Singapore among 227 children with food hypersensitivity confirmed that crustacean and fish are significant sensitizers in about 40 and 13% of children, respectively. Interestingly, the first intake of seafood seems to be very early in life in the Asian diet, with an average age of as low as 7 months. These findings were confirmed in a study on adults in Singapore [6] where 33% of patients were sensitized to crustaceans, followed by mollusks (19%) and fish (4%). Importantly, patients with fish or shellfish allergy, similar to peanut allergy, will mostly remain clinically reactive throughout their life. A follow-up study by Priftis *et al.* [7•] demonstrated that 65.5% of fish-sensitized children maintained their sensitization and are at increased risk for wheezing illness and hyperactive airways in school age.

Clinical features and diagnostic approaches of seafood allergy

The pattern of allergic symptoms after ingestion of seafood appears similar to those that occur during allergic reactions to other foods. However, similar symptom patterns are frequently reported by various contaminants of seafood (Table 1). Most reactions are immediate and reported within 2 h; however, late-phase reactions have been reported up to 8 h after ingestion of mollusks [8–10]. Patients may have a single symptom, but often there is a multiorgan involvement [6]. Particularly after ingestion of crustaceans, symptoms occur within minutes and include itching and angioedema of the lips, mouth and pharynx (oral allergy syndrome) [11•]. Shrimp has also been implicated in food-dependent exercise-induced anaphylaxis [12]. It seems that atopic individuals are at a higher risk of developing anaphylactic reactions.

In-vitro diagnostic methods include skin prick test (SPT) and quantification of specific IgE antibodies using assays such as the ImmunoCAP (Pharmacia, Uppsala, Sweden). Although skin testing for food allergy is often described as being problematic, a recent study by Jirapongsananuruk *et al.* [11•] convincingly demonstrated the high predictive probabilities using mean wheal diameters to determine positive food challenges with shrimp. A wheal diameter of 30 mm after SPT provided an 80 and 95% predictive probability of positive food challenge in patients allergic to the black tiger prawn (*Penaeus monodon*) and the giant fresh water prawn (*Macrobrachium rosenbergii*), respectively. Furthermore, monosensitivity to one of the other species could also be demonstrated using this test. SPT was also successfully correlated with positive food challenge by Carnes *et al.* [13] when using cooked instead of raw extracts of shrimps and lobster for skin testing. Furthermore, recent findings by Gill *et al.* [14] indicate that reactivity to certain allergens is associated with

Table 1 Adverse reactions to seafood generated by various substances

Etiology	Seafood implicated	Clinical symptoms	Time of onset
Bacterial			
Salmonella, Vibrio, Aeromonas, Listeria	Fish, crustacean, mollusk		
Viral			
Hepatitis A, rotavirus, astrovirus, small round viruses, etc.	Crustacean, mollusk		
Parasites			
Anisakis, Diphyllbothrium	All fish and cephalopods (e.g. squid)	Dermatological, oral allergy syndrome, gastrointestinal, neurological, respiratory	Minutes to several hours
Toxins			
Scombrotoxin	Fish, particularly with dark meat		
Ciguatera toxin	Reef fish		
Algae toxins	All mollusk species		
Allergens			
	Fish		
	Crustacean		
	Mollusk		
	Anisakis		

diseased risk. The gold standard for diagnosing food allergy is still the double-blind, placebo-controlled food challenge. A recent review by Niggemann and Beyer [15] proposes various decision trees to approach food challenges including seafood-allergic patients.

Allergens in the different seafood groups

The major allergen responsible for ingestion-related allergic reactions due to shellfish is the muscle protein, tropomyosin, in crustacean and mollusks. In addition to tropomyosin, other allergens have been identified and characterized in crustaceans such as the 40 kDa arginine kinase [16] (Table 2 [17–23,24^{••},25–27]), which might be a new class of invertebrate pan-allergens. A recent study by Ayuso *et al.* [24^{••}] demonstrated IgE binding to a 20 kDa allergen in 55% of patients reacting to the white pacific shrimp (*Litopenaeus vannamei*). The recombinant protein was subsequently produced and identified as a myosin light chain with high amino acid similarity to Bla g 8 from cockroaches. In addition, a sarcoplasmic calcium-binding protein was identified as allergen in black tiger shrimp [25] and tropomyosin in two krill species [26] and an amphipod [27].

Importantly, tropomyosin is not only a crustacean allergen but has been confirmed in a number of mollusk species, although mollusk allergens are not as well characterized as those of fish or crustacean. Nevertheless, it has become apparent that mollusks such as mussel, oyster, squid, limpet and abalone are significant food allergens in exposed population. In addition to tropomyosin [28], mollusks contain (Table 3 [29–31]) allergens such as myosin heavy chain, hemocyanin and amylase [32]. Even though IgE cross-reactivities are commonly reported, until recently, only limited work has been conducted on the molecular identity of these cross-reactive allergens. The amino acid sequence identity with shrimp tropomyosin is fairly low, with 57 and 61% (similar to nonallergenic vertebrate tropomyosin) for mussels and abalone, respectively, suggesting perhaps that other aller-

gens such as arginine kinase may be of more clinical relevance.

The first and most comprehensive study on any food allergen was the analysis of the major codfish allergen, Gad c 1, which belongs to a group of Ca-transporting muscle proteins known as parvalbumins [33[•]]. The molecular weight of parvalbumin, which ranges from 10 to 13 kDa, can be subdivided into two distinct isoforms, α and β . In fish, the allergenic β form is considered a cross-reactive pan-allergen. Although most heat-resistant food allergens contain linear epitopes, parvalbumin has conformational epitopes [34], stabilized by the interaction of metal-binding domains. Comparative studies of natural and recombinant allergens from cod and carp indicate that these proteins are equally good in the detection of parvalbumin-specific IgE [35[•]]. However, the need for strict quality control was highlighted along with differences in IgE antibody reactivity of different populations groups. This major allergen was recently also confirmed in four different tropical fish species [36] along with additional yet uncharacterized larger molecular allergens of 29 and 54 kDa. High-molecular-weight polymers of parvalbumin have previously been documented in snapper [37], but the clinical relevance is not known. Parvalbumin shares various degrees of amino acid homologies ranging from 60 to 80%, which probably explains variable degrees of clinical cross-reactivity in fish-allergic patients. In addition to parvalbumin, other allergens have been characterized in fish such as collagen [38], from the skin and muscle tissue, as well as the hormone, vitellogenin [39–42], found particularly in fish roe (caviar) (Table 4 [43–58]). It is of note that crustacean and mollusk allergens do not cross-react with fish allergens.

Allergic reactions to seafood are not always to the particular species-derived allergens. For example, contaminants such as the parasite *Anisakis*, if present, can cause severe allergic reactions [54]. The eight allergens from this parasite currently characterized include tropomyosin, which cross-reacts with shellfish allergens, as well as

Table 2 Allergenic proteins characterized in crustacean

Source of allergen	Allergen identified	Protein classification	Reference (2007–2008)
Chinese spiny lobster (<i>Panulirus stimpsoni</i>)	Pan s 1	Tropomyosin	[17]
American lobster (<i>Homarus americanus</i>)	Hom a 1	Tropomyosin	[17]
Red crab (<i>Charybdis feriatus</i>)	Cha f 1	Tropomyosin	[18]
Sand shrimp (<i>Metapenaeus ensis</i>)	Met e 1	Tropomyosin	[19]
Indian white shrimp (<i>Penaeus indicus</i>)	Pen i 1	Tropomyosin	[20]
Neptune rose shrimp (<i>Parapenaeus fissurus</i>)	Par f 1	Tropomyosin	[21]
Brown shrimp (<i>Penaeus aztecus</i>)	Pen a 1	Tropomyosin	[22]
Tiger shrimp (<i>Penaeus monodon</i>)	Pen m 2	Arginine kinase	[23]
White shrimp (<i>Litopenaeus vannamei</i>)	Lit v 2	Arginine Kinase	[16]
White shrimp (<i>Litopenaeus vannamei</i>)	Lit v 3	Myosin light chain	[24 ^{••}]
Tiger shrimp (<i>Penaeus monodon</i>)	–	Sarcoplasmic calcium-binding protein	[25]
Krill (<i>Euphausia superba</i>)	Eup s 1	Tropomyosin	[26]
Krill (<i>Euphausia pacifica</i>)	Eup p 1	Tropomyosin	[26]
Amphipods (<i>Gammarus spp.</i>); (<i>Caprella equilibra</i>)	–	Tropomyosin	[27]

Table 3 Allergenic proteins characterized in mollusks

Source of allergen	Allergen identified	Protein classification	Reference (2007–2008)
Japanese common squid (<i>Todarodes pacificus</i>)	Tod p 1	Tropomyosin	[29]
Pacific oyster (<i>Crassostrea gigas</i>)	Cra g 1, Cra g 2	Tropomyosin	[30]
Marine snail (<i>Turbo cornutus</i>)	Tur c 1	Tropomyosin	[31]
Abalone (<i>Haliotis midae</i>)	Hal m 1	Tropomyosin	[8]
Acorn barnacle (<i>Balanus rostratus</i>)	Bal r 1	Tropomyosin	[28]
Goose barnacle (<i>Capitulum mitella</i>)	Cap m 1	Tropomyosin	[28]

paramyosin and different protease inhibitors [54–58] (Table 4). A recent study demonstrated that these parasites could also cause allergic sensitization among fish-processing workers [59].

Cross-reactivity with other allergen sources

Patients with seafood allergy are frequently reported to also have allergic reactions to mites and insects [60]. This cross-reactivity is probably due to the high amino acid homology of these invertebrate tropomyosins and may have significant clinical implications. A recent study [61] demonstrated sensitization to shrimp tropomyosin in orthodox Jews who are prohibited by religious dietary laws from eating shellfish; however, as they were never exposed to crustacean allergens, this sensitization to tropomyosin is probably due to a noncrustacean source, such as house dust mites (HDM), cockroaches or both, via inhalation.

Clinically relevant cross-reactivity between crustacean and HDM allergens was described in the early 1990s by Wittenman *et al.* [62]. The primary sensitization is believed mostly to be ‘respiratory’ allergy to dust mites, which in some individuals may cause food-allergic reactions to crustaceans or mollusks. This view is supported by recent observations during immunotherapy to HDM in which some patients developed clinical sensitization to shellfish tropomyosin, which did not exist before therapy [63]. In contrast, sensitization to HDM in Iceland, where exposure to mites is extremely rare, is strongly correlated with specific IgE to shrimp [64]. Molecular studies suggest similar IgE-binding epitopes in these very different arthropods. Using synthetic overlapping peptides spanning the length of the major shrimp allergen, Pen a 1, eight IgE-binding epitopes were identified. Four out of eight epitopes are identical to homologous regions in Der p 10 and Der f 10 (from mites), and five out of eight are identical to homologous regions in Per a 7 (from

Table 4 Allergenic proteins characterized in fish

Source of allergen	Allergen identified	Protein classification	Reference (2007–2008)
Baltic cod (<i>Gadus callarias</i>)	Gad c 1	β-Parvalbumin	[43]
Atlantic salmon (<i>Salmo salar</i>)	Sal s 1	β-Parvalbumin	[44]
Carp (<i>Cyprinus carpio</i>)	Cyp c 1.01, Cyp c 1.02	β-Parvalbumin	[45]
Atlantic cod (<i>Gadus morhua</i>)	Gad m 1	β-Parvalbumin	[46]
Mackerel			[47]
<i>Scomber japonicus</i>	Sco j 1	β-Parvalbumin	
<i>Scomber australasicus</i>	Sco a 1	β-Parvalbumin	
<i>Scomber scombrus</i>	Sco s 1	β-Parvalbumin	
Alaska pollock (<i>Theragra chalcogramma</i>)	P1; P2	β-Parvalbumin	[48]
Pilchard (<i>Sardinops sagax</i>)	Sar sa 1.0101	β-Parvalbumin	[49]
Red snapper		Tetramer of parvalbumin	[37]
Golden snapper		Tetramer of parvalbumin	[37]
Big eye tuna		Collagen type I	[50]
Tuna, salmon, saurel, and mackerel skin		Gelatin	[51]
Cod	p41	Homologous to aldehyde phosphate dehydrogenase	[52]
Swordfish		ND	[53]
Bluefin tuna, blue marlin		Homologous to transferrin in red seabream fish	[51]
Trout caviar		Vitellogenin	[39]
Beluga fish caviar		Vitellogenin	[40]
Sea urchin roe			[41, 42]
Anisakis (<i>Anisakis simplex</i> ; <i>Anisakis pegreffii</i>)	Ani s 1	Kunitz-type serine protease inhibitor-like	[54–58]
	Ani s 2	Paramyosin	
	Ani s 3	Tropomyosin	
	Ani s 4	Cysteine protease inhibitor	
	Ani s 5	SXP/RAL protein	
	Ani s 6	Serine protease inhibitor	
	Ani s 7	Glycoprotein	
	Ani s 8	SXP/RAL protein	
	Ani s 9	SXP/RAL-2 family	

cockroach) [65]. These immunological findings strongly indicate that the documented cross-reactivity between tropomyosins from different allergen sources can result in IgE antibody binding and subsequent allergic reactions.

A multivariate statistical analysis of IgE antibody serum concentrations of 1011 individuals to 89 allergen extracts identified 12 stable clusters [66]. Interestingly, reactivity to shrimp and mussels was clustered with cockroach but unexpectedly not with HDM. This IgE cluster analysis suggests that mite tropomyosin (Der p 10) is not the major allergen and is probably based on the much lower amounts of this muscle protein in mites. A recent review on crustacean allergy highlights that in the group of arthropods, the crustaceans are phylogenetically closer related to the 'insects' than to the group of 'mites' (arachnids) (A.L. Lopata, S.B. Lehrer, unpublished observation). Other possible IgE reactivities to tropomyosin-containing allergen sources have been documented, such as the cross-reactivity with the nematode parasite, *Anisakis* [67], found in many seafood species. However, a low degree of cross-reactivity is also observed even between members of the crustacean family, such as barnacles, with amino acid sequence identity of only 61% when compared with that of shrimp [28]. A recent study by Marti *et al.* [68] used an in-silico motif-based allergenicity prediction protocol to generate a recombinant peptide that demonstrated the same IgE reactivity as shrimp full-length tropomyosin. This motif-generating algorithm may be used in the future to identify major IgE-binding structures of other coiled coil proteins.

Sensitization and provocation routes: gut, skin and lungs

It is well accepted that the major route of sensitization to food is through the gastrointestinal tract. This mechanism was confirmed for codfish allergens in animal [69] and human studies [70^{••}]. The use of antacid medication that increased the stomach pH can result in incomplete digestion and thereby increase exposure to and uptake of allergenic fish proteins or protein fragments. Challenge experiments on patients without clinical sensitivity demonstrated absorption of biologically active fish allergens already 10 min after ingestion. Fish digested at pH 3.0 revealed comparable reactivity patterns as undigested extracts. The increasing clinical reactions seen in fish-allergic patients after ingestion of partially digested fish proteins confirmed the quantitative absorption of fish allergens and elicitation of symptoms taking place most probably in the intestine.

The appearance of allergic symptoms results not only from ingestion of seafood but can also be triggered from inhaling cooking vapors and handling seafood in the domestic but particularly in the occupational setting

[71,72]. Symptoms manifest mainly as upper and lower airway respiratory symptoms and dermatitis, whereas anaphylaxis is rarely seen with this type of exposure. The prevalence of occupational asthma ranges from 7 to 36% and for occupational protein contact dermatitis, from 3 to 11% [73]. Airborne fish antigen exposures of more than 30 ng/m³ have recently shown significant correlation with sensitization and work-related asthma symptoms [71]. Exposure values among crustacean-processing environments are less well established, but exposures of about 3 mg/m³ seem to induce severe allergic reactions [74]. Not only sensitization but also reactions to food via the inhalational route seem to be common particularly at the workplace, as it is estimated that up to 15% of the asthmatic population in the USA and Europe have occupational asthma [75,76].

There is little information on the establishment of threshold values for elucidating allergic reactions to seafood. A recent study [70^{••}] demonstrated that for codfish, very small amounts of less than 3 mg protein could trigger allergic reactions, which is less than that previously reported [77].

For allergic reactions to crustacean, no new data have been established. Double-blind placebo-controlled studies with shrimp demonstrated allergic reactions after ingestion of at least two to four shrimps (4 g each, 16 g total) equivalent to 32 mg of extracted shrimp proteins [78]. Importantly, no fish or crustacean species has been found safe.

Treatment and management

Mutant tropomyosin from crustaceans was produced with reduced allergenic potency as demonstrated by rat basophil mediator release assays, and reduced activation was much more pronounced in the murine model [79].

Although the major IgE-binding epitopes of tropomyosin are linear, for the major fish allergen, parvalbumin, conformational epitopes are most probably relevant for IgE binding [34]. These antibody epitopes seem to be dependent on the functional reactivity of the binding sites for Ca²⁺ and Mg²⁺. Conformational changes in these helix-loop-helix metal-binding domains by side-directed mutagenesis can result in hypoallergenic parvalbumin as has been recently demonstrated for carp [80^{••}]. Although still immunogenic as demonstrated by IgG response in mice, the reactivity measured by SPT in patients is markedly reduced *in vivo*. This novel hypoallergenic protein is an important basis for safer novel forms of vaccination against fish allergy.

However, in general, management of food allergies, including seafood allergy, still relies mainly on avoidance.

The labeling of foods containing materials derived from seafood has already become mandatory in some countries such as the USA, Europe and Japan. Crustacean and fish have been previously recognized as important food allergens for labeling purposes (website: http://www.efsa.eu.int/science/nda/nda_opinions/catindex_en.html); however, the European Union guidelines were only recently adapted to include mollusks as a separate food allergen on the basis of the limited cross-reactivity to crustacean allergens (website: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178623594074.htm). In-vitro assays for the detection of crustacean tropomyosin are available, but cross-reactivities with other invertebrate tropomyosins from insects and mite are reported [81]. The detection of parvalbumin is much more problematic as these allergens show even higher biochemical and immunological variability among the different fish species [82].

Labeling regulations have limitations because of accidental cross-contamination with allergens through shared equipment in production lines or the unknown presence of a hidden allergen such as clarification agents derived from fish bladders used in wine and beer [83]. Support for the development of more specific and sensitive seafood allergen assays comes from a recent study on hidden food allergens in Spain. A retrospective study [84] on 530 adverse reactions to food established that the most common hidden allergen was the parasite *Anisakis*, causing 35% of allergic reactions in fish-allergic patients.

Conclusion

Although immunologic cross-reactivity between crustacean and mollusks is observed frequently, clinical cross-reactivity is not well studied. The often poor correlation of clinical symptoms with IgE reactivity to other arthropods such as HDM calls for improved in-vitro assays.

The increased repertoire of novel allergens using proteomics techniques and biotechnological production of allergens will improve the component-resolved diagnosis of seafood allergy. Improved reagent compositions will optimize the diagnosis for both clinical sensitivity and species specificity as well as accommodate geographical differences between populations. Moreover, the molecular approach will aid the development of novel specific immunotherapy for seafood allergy. However, the identification of specific diagnostic marker allergens, marker epitopes or both among the three major seafood groups, responsible for sensitization, clinical reactivity or both, will profoundly influence the management of allergic patients.

Whereas specific IgE assays have been so far mostly designed for single allergens, the increasing availability

of specific recombinant seafood allergens facilitates the possibilities for new applications. Recent developments in protein array technologies allow the simultaneous measurement of IgE reactivity to a large number of allergens.

Although these areas of technological developments are still in their infancies, it is likely that they will have a significant impact on more precise clinical implications for correct diagnosis, management and immunotherapy of seafood allergy.

Acknowledgements

This work was supported by grants from the Medical Research Council, the National Research Foundation (South Africa) and the Asthma Foundation Victoria (Australia) to Andreas L. Lopata and by the National Fisheries Institute to Samuel B. Lehrer.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Seafood consumption increases in 2006. In: National Oceanic and Atmospheric Administration. Administration NOAA. National Oceanic and Atmospheric Administration; 2007. <http://www.publicaffairs.noaa.gov/releases/2007/jul07/noaa07-r123.html>.
- 2 Ross MP, Ferguson M, Street D, *et al.* Analysis of food-allergic and anaphylactic events in the national electronic injury surveillance system. *J Allergy Clin Immunol* 2008; 121:166–171.
- 3 Vierk KA, Koehler KM, Fein SB, Street DA. Prevalence of self-reported food allergy in American adults and use of food labels. *J Allergy Clin Immunol* 2007; 119:1504–1510.
- A recent population-based prevalence study of food allergy among US adults and identification of difficulties in managing food allergy.
- 4 Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of seafood allergy in the United States determined by a random telephone survey. *J Allergy Clin Immunol* 2004; 114:159–165.
- 5 Chiang WC, Kidon MI, Liew WK, *et al.* The changing face of food hypersensitivity in an Asian community. *Clin Exp Allergy* 2007; 37:1055–1061.
- 6 Thong K, Suma Y, Ishizaki S, *et al.* Immediate food hypersensitivity among adults attending a clinical immunology/allergy centre in Singapore. *Singapore Med J* 2007; 48:236–240.
- 7 Priftis KN, Mermiri D, Papadopoulou A, *et al.* Asthma symptoms and bronchial reactivity in school children sensitized to food allergens in infancy. *J Asthma* 2008; 45:590–595.
- A case-control study among children with food allergy showed that most children retain their allergy to fish, and allergy to fish in infants is an increased risk for wheezing illness and hyperactive airways in school age.
- 8 Lopata AL, Zinn C, Potter PC. Characteristics of hypersensitivity reactions and identification of a unique 49 kd IgE-binding protein (Hal-m-1) in abalone (*Haliotis midae*). *J Allergy Clin Immunol* 1997; 100:642–648.
- 9 Villacis J, Rice TR, Bucci LR, *et al.* Do shrimp-allergic individuals tolerate shrimp-derived glucosamine? *Clin Exp Allergy* 2006; 36:1457–1461.
- 10 Wild LG, Lehrer SB. Fish and shellfish allergy. *Curr Allergy Asthma Rep* 2005; 5:74–79.
- 11 Jirapongsananuruk O, Sripramong C, Pacharn P, *et al.* Specific allergy to *Penaeus monodon* (seawater shrimp) or *Macrobrachium rosenbergii* (freshwater shrimp) in shrimp-allergic children. *Clin Exp Allergy* 2008; 38:1038–1047.
- This study convincingly demonstrated the high predictive probability using SPT as well as prick-to-prick testing. Furthermore, species-specific reactions to different shrimps were demonstrated by food challenge.
- 12 Zhang Y, Matsuo H, Morita E. Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy. *J Dermatol* 2006; 33:174–177.

- 13 Carnes J, Ferrer A, Huertas AJ, *et al*. The use of raw or boiled crustacean extracts for the diagnosis of seafood allergic individuals. *Ann Allergy Asthma Immunol* 2007; 98:349–354.
- 14 Gill BV, Cartier A, Horth-Susin L, *et al*. IgE antibody responses to snow crab proteins in snow crab processing workers. *J Invest Med* 2003:S244.
- 15 Niggemann B, Beyer K. Diagnosis of food allergy in children: toward a standardization of food challenge. *J Pediatr Gastroenterol Nutr* 2007; 45:399–404.
- 16 Garcia-Orozco KD, Aispuro-Hernandez E, Yepiz-Plascencia G, *et al*. Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. *Int Arch Allergy Immunol* 2007; 144:23–28.
- 17 Leung PSC, Chen YC, Mykles DL, *et al*. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Mol Mar Biol Biotechnol* 1998; 7:12–20.
- 18 Leung PSC, Chen YC, Gershwin ME, *et al*. Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *J Allergy Clin Immunol* 1998; 102:847–852.
- 19 Leung PSC, Chu KH, Chow WK, *et al*. Cloning, expression, and primary structure of metapenaeus-ensis tropomyosin, the major heat-stable shrimp allergen. *J Allergy Clin Immunol* 1994; 94:882–890.
- 20 Shanti KN, Martin BM, Nagpal S, *et al*. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J Immunol* 1993; 151:5354–5363.
- 21 Lin RY, Shen HD, Han SH. Identification and characterization of a 30-Kd major allergen from *Parapenaeus fissurus*. *J Allergy Clin Immunol* 1993; 92:837–845.
- 22 Daul CB, Slattery M, Reese G, Lehrer SB. Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *Int Arch Allergy Immunol* 1994; 105:49–55.
- 23 Yu CJ, Lin YF, Chiang BL, Chow LP. Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 2003; 170:445–453.
- 24 Ayuso R, Grishina G, Bardina L, *et al*. Myosin light chain is a novel shrimp allergen, Lit v 3. *J Allergy Clin Immunol* 2008; 122:795–802.
This comprehensive study demonstrates not only the identification of a novel shrimp allergen but also describes the molecular analysis and immunological characterization of the recombinant allergen produced.
- 25 Shiomi K, Sato Y, Hamamoto S, *et al*. Sarcoplasmic calcium-binding protein: identification as a new allergen of the black tiger shrimp *Penaeus monodon*. *Int Arch Allergy Immunol* 2008; 146:91–98.
- 26 Nakano S, Yoshinuma T, Yamada T. Reactivity of shrimp allergy-related IgE antibodies to krill tropomyosin. *Int Arch Allergy Immunol* 2008; 145:175–181.
- 27 Motoyama K, Hamada Y, Nagashima Y, Shiomi K. Allergenicity and allergens of amphipods found in nori (dried laver). *Food Addit Contam* 2007; 24:917–922.
- 28 Suma Y, Ishizaki S, Nagashima Y, *et al*. Comparative analysis of barnacle tropomyosin: divergence from decapod tropomyosins and role as a potential allergen. *Comp Biochem Physiol B Biochem Mol Biol* 2007; 147:230–236.
- 29 Miyazawa H, Fukamachi H, Inagaki Y, *et al*. Identification of the first major allergen of a squid (*Todarodes pacificus*). *J Allergy Clin Immunol* 1996; 98:948–953.
- 30 Ishikawa M, Ishida M, Shimakura K, *et al*. Tropomyosin, the major oyster *Crassostrea gigas* allergen and its IgE-binding epitopes. *J Food Sci* 1998; 63:44–47.
- 31 Ishikawa M, Ishida M, Shimakura K, *et al*. Purification and IgE-binding epitopes of a major allergen in the gastropod *Turbo cornutus*. *Biosci Biotechnol Biochem* 1998; 62:1337–1343.
- 32 Taylor SL. Molluscan shellfish allergy. *Adv Food Nutr Res* 2008; 54:139–177.
- 33 Jenkins JA, Breiteneder H, Mills ENC. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *J Allergy Clin Immunol* 2007; 120:1399–1405.
A very informative investigation of structural relationships of tropomyosin and parvalbumin, their evolutionary relatedness to human homologs and consideration for immunotherapy.
- 34 Untermayr E, Szalai K, Riemer AB, *et al*. Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Mol Immunol* 2006; 43:1454–1461.
- 35 Ma Y, Griesmeier U, Susani M, *et al*. Comparison of natural and recombinant forms of the major fish allergen parvalbumin from cod and carp. *Mol Nutr Food Res* 2008; 52:196–207.
This comprehensive study compares the molecular and immunological characteristics of natural with recombinant forms of parvalbumin and highlights potential problems.
- 36 Lim DLC, Neo KH, Yi FC, *et al*. Parvalbumin: the major tropical fish allergen. *Pediatr Allergy Immunol* 2008; 19:399–407.
- 37 Rosmilah M, Shahnaz M, Masita A, *et al*. Identification of major allergens of two species of local snappers: *Lutjanus argentimaculatus* (merah/red snapper) and *Lutjanus johnii* (jenahak/golden snapper). *Trop Biomed* 2005; 22:171–177.
- 38 Sakaguchi M, Toda M, Ebihara T, *et al*. IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *J Allergy Clin Immunol* 2000; 106:579–584.
- 39 Escudero R, Gamboa PM, Anton J, Sanz ML. Food allergy due to trout roe. *J Investig Allergol Clin Immunol* 2007; 17:346–347.
- 40 Perez-Gordo M, Sanchez-Garcia S, Cases B, *et al*. Identification of vitellogenin as an allergen in Beluga caviar allergy. *Allergy* 2008; 63:479–480.
- 41 Hickey RW. Sea urchin roe (uni) anaphylaxis. *Ann Allergy Asthma Immunol* 2007; 98:493–494.
- 42 Rodriguez V, Bartolome B, Armisen M, Vidal C. Food allergy to *Paracentrotus lividus* (sea urchin roe). *Ann Allergy Asthma Immunol* 2007; 98:393–396.
- 43 Elsayed S, Apold J. Immunochemical analysis of cod fish allergen-M: locations of the immunoglobulin binding-sites as demonstrated by the native and synthetic peptides. *Allergy* 1983; 38:449–459.
- 44 Lindstrom CDV, VanDo T, Hordvik I, *et al*. Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic salmon (*Salmo salar*). *Scand J Immunol* 1996; 44:335–344.
- 45 Swoboda I, Bugajska-Schretter A, Valenta R, Spitzauer S. Recombinant fish parvalbumins: candidates for diagnosis and treatment of fish allergy. *Allergy* 2002; 57:94–96.
- 46 Van Do T, Hordvik I, Endresen C, Elsayed S. The major allergen (parvalbumin) of codfish is encoded by at least two isotypic genes: cDNA cloning, expression and antibody binding of the recombinant allergens. *Mol Immunol* 2003; 39:595–602.
- 47 Hamada Y, Tanaka H, Ishizaki S, *et al*. Purification, reactivity with IgE and cDNA cloning of parvalbumin as the major allergen of mackerels. *Food Chem Toxicol* 2003; 41:1149–1156.
- 48 Van Do T, Hordvik I, Endresen C, Elsayed S. Characterization of parvalbumin, the major allergen in Alaska pollack, and comparison with codfish allergen M. *Mol Immunol* 2005; 42:345–353.
- 49 Beale J, Jeebhay MF, Lopata AL. Characterisation of purified parvalbumin from five fish species and nucleotide sequencing of the major allergen from Pacific pilchard, *Sardinops sagax*. (in press).
- 50 Hamada Y, Nagashima Y, Shiomi K. Identification of collagen as a new fish allergen. *Biosci Biotechnol Biochem* 2001; 65:285–291.
- 51 Taylor SL, Kabourek JL, Hefle SL. Fish allergy: fish and products thereof. *J Food Sci* 2004; 69:R175–R180.
- 52 Galland AV, Dory D, Pons L, *et al*. Purification of a 41 kDa cod-allergenic protein. *J Chromatogr B* 1998; 706:63–71.
- 53 Kelso JM, Jones RT, Yunginger JW. Monospecific allergy to swordfish. *Ann Allergy Asthma Immunol* 1996; 77:227–228.
- 54 Audicana MT, Kennedy MW. Anisakis simplex: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev* 2008; 21:360–379.
- 55 Rodriguez-Perez R, Caballero ML, Gonzalez-Munoz M, *et al*. Cloning and expression of a biologically active *Anisakis simplex* allergen Ani s 1 in the yeast *Pichia pastoris*. *Mol Biochem Parasitol* 2007; 154:115–118.
- 56 Rodriguez-Perez R, Moneo I, Rodriguez-Mahillo A, Caballero ML. Cloning and expression of Ani s 9, a new *Anisakis simplex* allergen. *Mol Biochem Parasitol* 2008; 159:92–97.
- 57 Rodriguez-Mahillo AI, Gonzalez-Munoz M, Gomez-Aguado F, *et al*. Cloning and characterisation of the *Anisakis simplex* allergen Ani s 4 as a cysteine-protease inhibitor. *Int J Parasitol* 2007; 37:907–917.
- 58 Kobayashi Y, Ishizaki S, Nagashima Y, Shiomi K. Ani s 1, the major allergen of *Anisakis simplex*: purification by affinity chromatography and functional expression in *Escherichia coli*. *Parasitol Int* 2008; 57:314–319.
- 59 Nieuwenhuizen N, Lopata AL, Jeebhay MLF, *et al*. Exposure to the fish parasite *Anisakis* causes allergic airway hyperreactivity and dermatitis. *J Allergy Clin Immunol* 2006; 117:1098–1105.
- 60 Ayuso R, Reese G, Leong-Kee S, *et al*. 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.
- 61 Fernandes J, Reshef A, Patton L, *et al*. Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, in unexposed orthodox Jews. *Clin Exp Allergy* 2003; 33:956–961.
- 62 Witterman AM, Akkerdaas JH, Vanleeuwen J, *et al*. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol* 1994; 105:56–61.

- 63 vanRee R, Antonicelli L, Akkerdaas JH, *et al*. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996; 51:108–113.
- 64 Adalsteinsdottir B, Sigurdardottir ST, Gislason T, *et al*. What characterizes the house dust mite sensitive individuals in a house dust mite free community in Reykjavik, Iceland? *Allergol Int* 2007; 56:51–56.
- 65 Reese G, Ayuso R, Leong-Kee SM, *et al*. Epitope mapping and mutational substitution analysis of the major shrimp allergen Pen a 1 (tropomyosin). *J Allergy Clin Immunol* 2002; 109:S307–S1307.
- 66 Soeria-Atmadja D, Onell A, Kober A, *et al*. Multivariate statistical analysis of large-scale IgE antibody measurements reveals allergen extract relationships in sensitized individuals. *J Allergy Clin Immunol* 2007; 120:1433–1440.
- 67 Jeong KY, Hong CS, Yong TS. Allergenic tropomyosins and their cross-reactivities. *Protein Pept Lett* 2006; 13:835–845.
- 68 Marti P, Truffer R, Stadler MB, *et al*. Allergen motifs and the prediction of allergenicity. *Immunol Lett* 2007; 109:47–55.
- 69 Pali-Scholl I, Yildirim AO, Ackermann U, *et al*. Antiacids lead to immunological and morphological changes in the intestine of BALB/c mice similar to human food allergy. *Exp Toxicol Pathol* 2008; 60:337–345.
- 70 Untersmayr E, Vestergaard H, Malling HJ, *et al*. Incomplete digestion of codfish represents a risk factor for anaphylaxis in patients with allergy. *J Allergy Clin Immunol* 2007; 119:711–717.
- This is an excellent study that assesses the absorption kinetics of fish proteins, investigates the clinical reactivity of patients with fish allergy to codfish digested at different pH and confirms the risk of severe allergic reactions to minute amounts of allergens.
- 71 Jeebhay MF, Robins TG, Malo JL, *et al*. Occupational allergy and asthma among salt water bony-fish processing workers. *Am J Ind Med* 2008; 51:899–910.
- 72 Jeebhay MF, Robins TG, Seixas N, *et al*. Environmental exposure characterization of fish processing workers. *Ann Occup Hyg* 2005; 49:423–437.
- 73 Jeebhay MF, Robins TG, Lehrer SB, Lopata AL. Occupational seafood allergy: a review. *Occup Environ Med* 2001; 58:553–562.
- 74 Arts JHE, Mommers C, de Heer C. Dose-response relationships and threshold levels in skin and respiratory allergy. *Crit Rev Toxicol* 2006; 36:219–251.
- 75 Bardana EJ. Occupational asthma. *J Allergy Clin Immunol* 2008; 121:S408–S411.
- 76 Jeebhay MF, Quirce S. Occupational asthma in the developing and industrialised world: a review. *Int J Tuberc Lung Dis* 2007; 11:122–133.
- 77 Taylor SL, Hefle SL, Bindslev-Jensen C, *et al*. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002; 109:24–30.
- 78 Daul CB, Morgan JE, Hughes J, Lehrer SB. Provocation-challenge studies in shrimp-sensitive individuals. *J Allergy Clin Immunol* 1988; 81:1180–1186.
- 79 Reese G, Viebranz J, Leong-Kee SM, *et al*. Reduced allergenic potency of VR9-1, a mutant of the major shrimp allergen Pen a 1 (tropomyosin). *J Immunol* 2005; 175:8354–8364.
- 80 Swoboda I, Bugajska-Schretter A, Linhart B, *et al*. A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. *J Immunol* 2007; 178:6290–6296.
- This interesting study demonstrates the production of hypoallergenic parvalbumin by conformational changes of this protein. Although still immunogenic in mice, this novel hypoallergenic protein could form the basis for new forms of vaccination against fish allergy.
- 81 Sakai S, Matsuda R, Adachi R, *et al*. Interlaboratory evaluation of two enzyme-linked immunosorbent assay kits for the determination of crustacean protein in processed foods. *J AOAC Int* 2008; 91:123–129.
- 82 Faeste CK, Plassen C. Quantitative sandwich ELISA for the determination of fish in foods. *J Immunol Methods* 2008; 329:45–55.
- 83 Rolland JM, Apostolou E, Deckert K, *et al*. Potential food allergens in wine: double-blind, placebo-controlled trial and basophil activation analysis. *Nutrition* 2006; 22:882–888.
- 84 Anibarro B, Seoane FJ, Mugica MV. Involvement of hidden allergens in food allergic reactions. *J Investig Allergol Clin Immunol* 2007; 17:168–172.