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Allergens and Molecular Diagnostics of Shellfish Allergy

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21.1 Background

In recent years, there has been a steady growth in the production and consumption of seafood and partial shellfish. This increased consumption has led to an increase in adverse health problems among consumers including allergic reactions.

The pattern of allergic symptoms after ingestion of crustaceans appears similar to the symptoms experienced due to other foods. Reactions are immediate and reported mostly within 2 hours; however, late-phase reactions have been reported up to 8 hours after ingestion, particularly to snow crab, cuttlefish, limpet, and abalone (Lopata et al. 1997; Villacis et al. 2006). Patients may have a single symptom but often there is a multi-organ involvement. Importantly, respiratory reactions are often

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seen after ingestion of allergenic seafood and frequently anaphylactic reactions (Matricardi et al. 2016). Particularly, the oral allergy syndrome (OAS) seems to be very often experienced by crustacean allergic subjects. Shrimp has also been implicated in food-dependent exercise-induced anaphylaxis (Zhang et al. 2006).

Currently, 2% of the general world population is affected by shellfish allergy, with much higher rates in countries with high seafood consumption. Unlike many other food allergies, most shellfish allergy persists for life in the affected individual.

21.2 Classification of Shellfish Groups

Patients with allergy to shellfish may fail to identify the offending seafood species, often as a result of confusion regarding the different common names used to describe diverse seafood. The two invertebrate phyla of arthropods and mollusks are generally referred to as "shellfish" (see ◉ Fig. 21.1).

Crustaceans are, perhaps surprisingly, classified as arthropods together with spiders and insects. This might provide an explanation for the observed molecular and clinical cross-reactivity discussed in detail below. Over 30,000 living crustacean

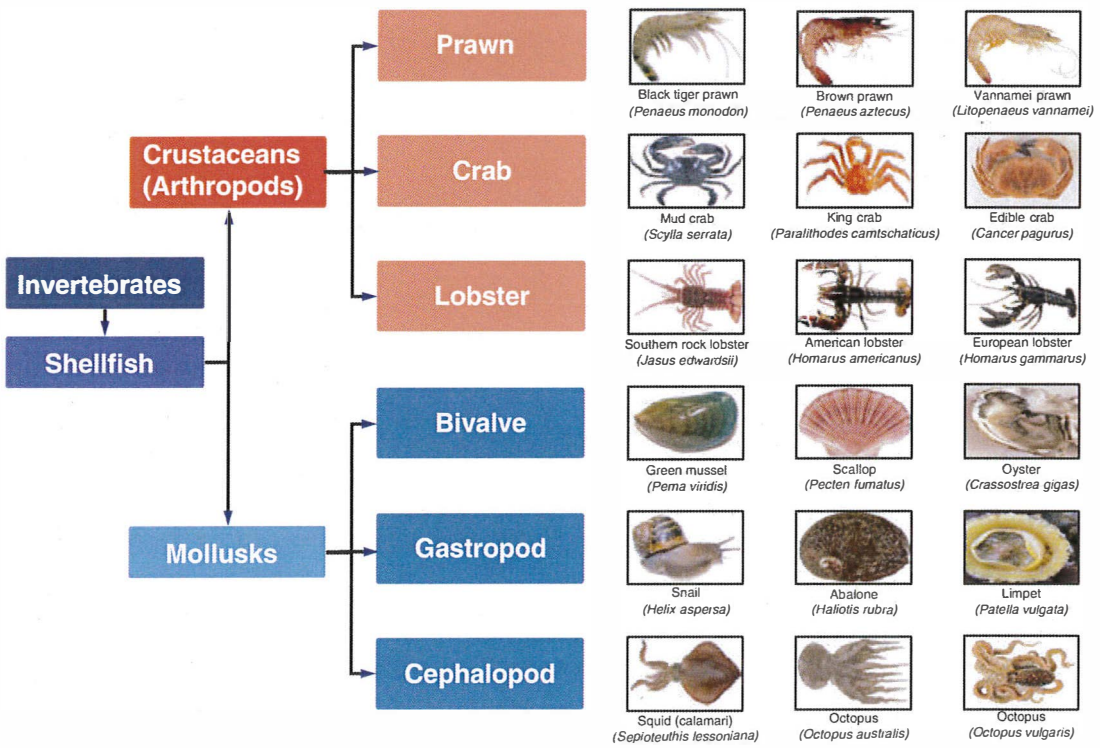

Fig. 21.1 Schematic classification of most commonly consumed shellfish species

species are found worldwide, and large varieties are consumed raw or cooked worldwide.

The group of mollusks is a large and diverse group, subdivided into the classes' bivalve, gastropod and cephalopod. This group comprises over 100,000 different species, including several important seafood groups including mussels, oysters, abalone, snails, and squid (calamari).

21.3 Prevalence of Shellfish Allergy

The prevalence of allergic reactions to seafood is usually higher when the consumption plays a greater part in the diet of the observed community (see ◉ Table 21.1) (Lopata et al. 2016). It is generally considered that crustacean and mollusk are among the foods that most commonly provoke severe anaphylaxis (Tham et al. 2008). A recent study established surprisingly that seafood allergies are a significant health concern affecting approximately 6.5 million people in the USA – more than twice as common as peanut allergy. The telephone survey among 14,948 individuals reported 5.9 % with shellfish allergy, and seafood allergy was almost five times more common among adults compared to children. Of all the subjects with allergies to crustacean and mollusk, only 38 % and 49 %, respectively, reported reactions to multiple species, and only 14 % reacted to both shellfish groups (Sicherer et al. 2004).

In France, a study by Andre and co-worker among 580 patients with adverse reactions to food, 34 % were identified having specific IgE to crab (Andre et al. 1994). A study by Crespo et al. in Spain established that 6.8 % of patients reacted to crustaceans (Crespo et al. 1995). A study from South Africa including 105 individuals with perceived adverse reactions to seafood confirmed sensitization to shrimps and rock lobster in almost 50 % (Lopata et al. 1997; Zinn et al. 1997).

While seafood allergy is common in Western countries such as Europe, the USA, and Australia, it seems that in Asian countries, allergic reactions to shellfish are of greater importance among adults and children (Goh et al. 1999; Shek et al. 2010; Thalayasingam et al. 2015). This clearly supports the view that the likelihood of becoming sensitized to shellfish seems to correlate with geographical eating habits and is most likely underreported in many Asian populations.

Not only ingestion of shellfish can cause sensitization but also exposure during processing in factories and domestic environment. There seems to be a strong correlation between high concentration of airborne allergens and increased allergic sensitization (Baatjies et al. 2015; Kamath et al. 2014a). Crustaceans seem to produce the strongest allergic response during processing of seafood and reach prevalence rates of up to 30 % (Bonlokke et al. 2012; Gautrin et al. 2010).

Table 21.1 List of identified and characterized shellfish allergens according to the International Union of Immunological Societies (IUIS) allergen nomenclature

	Biochemical name	Molecular weight	Heat stability and IgE binding	Route of exposure	IgE sensitization (%) (n = subjects tested)	Physiological function
1	Tropomyosin	34–38 kDa	Highly heat stable and IgE reactive	Ingestion Inhalation	Pen a 1, 51 % (n = 45) Gámez et al. (2011) Lit v 1, 61 % (n = 19) Ayuso et al. (2010) Pen m 1, 62 % (n = 16) Kamath et al. (2014b)	Coiled-coil protein that binds to actin and regulates interaction of troponin and myosin
2	Arginine kinase	40–45 kDa	Labile but can elicit IgE binding	Ingestion Inhalation	Pen m 2, 50 % (n = 16) Kamath et al. (2014b) Lit v 2, 21 % (n = 19) Ayuso et al. (2010)	A kinase that catalyzes reversible transfer of phosphoryl group from ATP to arginine
3	Myosin light chain	17–20 kDa	Stable	Ingestion	Pen m 3, 31 % (n = 16) Kamath et al. (2014b) Lit v 3, 31 % (n = 19) Ayuso et al. (2010)	Regulatory function in smooth muscle contraction when phosphorylated by MLC kinase
4	Sarcoplasmic calcium-binding protein	20–25 kDa	Stable	Ingestion	Pen m 4, 19 % (n = 16) Kamath et al. (2014b) Lit v 4, 21 % (n = 19) Ayuso et al. (2010)	Binds to cytosolic calcium (Ca^{2+}) and acts as a calcium buffer regulating calcium-based signaling
5	Troponin C	20–21 kDa	Unknown	Ingestion	Cra c 6, 29 % (n = 31) Bauermeister et al. (2011)	Regulates interaction of actin and myosin during muscle contraction on binding to calcium
6	Triose-phosphate isomerase	28 kDa	Labile	Ingestion Inhalation	Pen m 8, 19 % (n = 16) Kamath et al. (2014b) Cra c 8, 23 % (n = 31) Bauermeister et al. (2011)	Key enzyme in glycolysis; catalyzes conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate

21.4 Structure and Biological Functions of Shellfish Allergens

Over the past 20 years, several shellfish allergens, particularly in crustaceans, have been identified and sequenced (◉ Table 21.2). Currently, 34 allergens have been identified and characterized in detail from various crustacean and mollusk species and registered with the International Union of Immunological Societies (IUIS) Allergen Database (Radauer et al. 2008). Most of these allergens belong to six different protein families. The biochemical characteristics of shellfish allergenic proteins are typically low molecular weight, high water solubility, high heat stability, and an acidic isoelectric point. Almost all of the known characterized allergens are found in the edible portions of various shellfish species. For example, the major shellfish allergen tropomyosin is found in the abdominal part of prawns, pincer and tail of crabs and lobsters, as well as body or arm/tentacles of octopus and squid. However, some protease-based allergens, which cause clinical reactions through the protease-activated receptor (PAR) pathway (non-IgE mediated), are present in the gastrointestinal regions of the different shellfish species (Sun and Lopata 2010). The allergen family-specific properties of shellfish allergens are described below (see ◉ Table 21.1):

1. *Tropomyosin (TM) Pen m 1*

   Tropomyosin is the major allergenic protein across all edible crustacean and mollusk species. It is also the most abundant allergen in shellfish, constituting up to 20% of the total protein. More than 60% of shellfish-allergic patients are sensitized and react to TM, often leading to severe systemic reactions. Tropomyosin-specific IgE is frequently used to predict clinical outcomes of shrimp allergy with a positive predictive value of 0.72 (Gámez et al. 2011; Pascal et al. 2015).

   Tropomyosin forms a large family of proteins, which are associated to actin filaments and play a critical role in the regulation of actin filaments in muscle and non-muscle cells (Oguchi et al. 2011). This allergen is an alpha-helical coiled-coil dimeric protein that binds along the length of actin and regulates the cooperation of troponin and myosin, thus controlling the contraction of muscle fibers (Oguchi et al. 2011). Due to TM's primary role in muscle contraction regulation, the primary structure is highly conserved across various invertebrate species. This seems the main reason for high IgE-mediated allergenic cross-reactivity across various shellfish species as described below in detail. Depending on alternate splicing mechanisms, different isoforms of tropomyosin are generated, with structural and functional differences (Reese et al. 1999). In crustacean species, the fast twitch and the slow twitch isoforms were identified in the tail and pincer muscles, respectively (Motoyama et al. 2007). Interestingly, even though crustacean and mollusk tropomyosins are allergenic, they share only very low amino acid sequence identities of 55–70%.

   Allergenic TMs have generally molecular weights of between 33 kDa and 38 kDa and are highly stable to heat treatment, capable of retaining allergenicity

Table 21.2 Characterized allergens in crustacean and mollusk species

	Shellfish species	Common names	Tropomyosin	Arginine kinase	Myosin light chain 1 and 2	Sarcoplasmic calcium-binding protein	Troponin C, troponin I	Triose-phosphate isomerase
Prawn	*Penaeus monodon*	Black tiger prawn, giant tiger prawn, Asian tiger shrimp	Pen m 1[b]	Pen m 2[b]	Pen m 3	Pen m 4[b]	Pen m 6	Cra c 8
	Penaeus aztecus	Brown shrimp	Pen a 1[a]	–	–	–	–	–
	Crangon crangon	North Sea shrimp, common shrimp	Cra c 1	Cra c 2	Cra c 5	Cra c 4	Cra c 6	–
	Litopenaeus vannamei	Pacific white shrimp, vannamei shrimp	Lit v 1	Lit v 2	Lit v 3	Lit v 4	–	–
	Melicertus latisulcatus	King prawns, Western king prawns	Mel l 1	–	–	–	–	–
	Pandalus borealis	Northern shrimp, pink shrimp	Pan b 1	–	–	–	–	–
	Penaeus indicus	Indian white prawn	Pen i 1	–	–	–	–	–
	Metapenaeus ensis	Greasyback shrimp, sand shrimp	Met e 1	–	–	–	–	–
	Archaeopotamobius sibiriensis	ND	–	–	–	–	–	Arc s 8
Crab	*Charybdis feriatus*	Crucifix crab	Cha f 1	–	–	–	–	–
	Portunus pelagicus	Blue swimmer crab	Por p 1	–	–	–	–	–
Lobster	*Homarus americanus*	American lobster	Hom a 1		Hom a 3		Hom a 6	
	Panulirus stimpsoni	Spiny lobster	Pan s 1	–	–	–	–	–
	Pontastacus leptodactylus	Narrow-clawed crayfish	Pon i 1	–	–	–	–	–
Bivalve	*ND*		–	–	–	–	–	–
Gastropod	*Helix aspersa*	Garden snail	Hel as 1	–	–	–	–	–
	Haliotis midae	South African abalone	Hal m 1	–	–	–	–	–
Cephalopod	*Todarodes pacificus*	Pacific squid	Tod p 1	–	–	–	–	–

Allergens stated are registered with the IUIS allergen nomenclature
"–"and "ND" indicates not determined
[a]Allergens included in ImmunoCAP
[b]Allergens included in ISAC

even after cooking and high-pressure processing. However, some studies have demonstrated modulation of IgE recognition to tropomyosin due to heat-induced Maillard reaction, which may occur in some shellfish species (Nakamura et al. 2005, 2006.

According to the AllFam database, the TM family is the largest "food" allergen family in animal sources, consisting of currently 47 identified TMs, mostly from crustacean species (Radauer et al. 2008). Examples of well-characterized TM are Pen m 1, Pen a 1, Lit v 1, and Hom a 1.

2. *Arginine kinase* (*AK*) *Pen m 2*

Arginine kinase was first characterized as an allergen in Indian meal moth (Binder et al. 2001). Since then, AK has been identified in over six crustacean and one mollusk species. Arginine kinase belongs to a class of kinases that catalyze the reversible transfer of the high-energy phosphoryl group from ATP to arginine, thus yielding ADP and *N*-phosphoarginine (Yu et al. 2003). These phosphagens then serve as high energy source from which ATP can be replenished in many invertebrate species (Pereira et al. 2000). Creatinine kinase serves this purpose in higher vertebrates.

IgE sensitization to AK has been demonstrated in 21–50 % of adults and 67 % of children (Kamath et al. 2014b; Yang et al. 2010). However, the frequency of clinical reactivity to AK has not been investigated in detail. Invertebrate AK has a molecular weight of 40–42 kDa and is not stable to acid or alkali treatment. Unlike tropomyosin, AK is also not stable to heat treatment. However, IgE binding has been demonstrated to AK in heat-treated shrimps, which may be due to remaining intact IgE epitopes on aggregated AK (Kamath et al. 2014b; Shen et al. 2012). Interestingly, crustacean AK along with TM has also been implicated in inhalational exposure and sensitization among crab-processing workers (Abdel Rahman et al. 2011). Crustacean AK has been demonstrated to cross-react to ingested insect AK as well as being implicated in seafood-mite cross-reactivity (Srinroch et al. 2015; Gamez et al. 2014).

3. *Myosin light chain* (*MLC*) *Pen m 3*

The EF-hand domain superfamily is the second largest group of all allergens, after profilins, which encompasses both food and inhalant allergens from animal and plant sources. Three classes of shellfish allergens are EF-hand domain proteins, which include MLC, sarcoplasmic calcium-binding proteins, and troponin. Interestingly, the major allergen in fish is parvalbumin, which is also an EF-hand domain allergen.

MLC is mainly found in smooth muscles in complex with myosin heavy chain motor domains. During muscle contraction, the calcium-calmodulin complex, MLC kinase is activated, which in turn phosphorylates myosin light chain, regulating the smooth muscle movement (Kamm and Stull 1985). Two isoforms are currently known, the essential MLC and regulatory MLC. As an EF-hand domain protein, the regulatory MLC binds metal ions, mostly with magnesium (Trybus 1994). Myosin light chains have a molecular weight between 17 and 20 kDa, are well characterized in four crustacean species, and seem to be heat stable. Currently, there is a lack of data on immunological cross-reactivity of

MLC among crustaceans, mollusks, or other invertebrate species. An amino acid sequence alignment for MLC based on sequences available on GenBank estimates an identity ranging between 86 and100 %; although this is highly dependent on the isoforms sequenced.

4. *Sarcoplasmic calcium-binding protein* (*SCBP*) *Pen m 4*
   Sarcoplasmic calcium-binding proteins are also members of the EF-hand calcium-binding protein family incorporating the helix-loop-helix motif in the primary amino acid sequence. It is believed to function as the invertebrate counterpart of vertebrate parvalbumin. Its main activity is the regulation of the cytosolic calcium (Ca2+) concentration, thus assisting in calcium-dependent cell signaling. SCBP is ubiquitously expressed throughout the organism, but more abundant in the abdominal muscle (Gao et al. 2006). In mollusks, it is located in a tissue-specific manner (Hermann and Cox 1995). It has a molecular weight of approximately 20 kDa and an isoelectric point of 5 and can elicit IgE binding even after heat treatment (Kamath et al. 2014b). Due to its similar molecular weight with that of MLC, it is difficult to establish the IgE recognition pattern using traditional immunochemical methods such as immunoblotting. Recent studies have highlighted the relevance of SCBP as a shellfish allergen. Ayuso et al. demonstrated IgE recognition in 85 % of shrimp-allergic children, which is much higher compared to tropomyosin (Ayuso et al. 2009). More importantly, it has been shown that specific IgE to SCBP, in addition to that of TM, is associated with clinical reactivity to shrimps (Pascal et al. 2015).
5. *Troponin C* (*TnC*) *Cra c 6*
   Troponin C has been characterized in shrimps, but also as important cockroach allergen (Bla g 6 and Per a 6). Similar to SCBP and MLC, TnC is an EF-hand calcium-binding protein. Troponin C forms a complex with troponin I and TM. Based on conformational changes to the complex, due to calcium influx, it regulates the interaction of actin and myosin during muscle contraction (Hindley et al. 2006). Troponin C is approximately 20 kDa in size and its possible heat stability is not fully understood. Interestingly, it was demonstrated that IgE binding to Bla g 6 (cockroach) increased after addition of calcium in previously depleted serum, indicating the possible presence of calcium-dependent conformational IgE epitopes on TnC. The IgE-binding frequency to TnC is with 15 % lower as reactivity to TM, AK, or SCBP.
6. *Triose-phosphate isomerase* (*TIM*) *Cra c 8*
   Triose-phosphate isomerase plays an important role in the glycolysis involved in energy production. TIM catalyzes the conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate, the final product of this metabolic pathway being pyruvate. This allergen has been characterized in shrimps (Cra c 8), crayfish (Arc s 8), and cockroach (Bla g TPI). It has an approximate molecular weight of 28 kDa and is probably heat sensitive (Bauermeister et al. 2011). The clinical and immunological cross-reactivity of TIM among various invertebrate species are not well understood and amino acid sequences have not been performed.

21.5 Clinical and Immunological Cross-Reactivity

True sensitization to shellfish-specific allergens can be hampered due the highly cross-reactive nature of some allergenic proteins. The best-known panallergen is tropomyosin, being the major cause for reported clinical cross-reactivity among and between crustacean and mollusk, but also other invertebrates, including mites, cockroaches, and parasites (see ◉ Fig. 21.2). Some conserved regions of IgE-binding epitope of tropomyosin seem to be shared between crustaceans and mollusks. It is known that tropomyosin has mainly linear IgE epitopes and is of great importance in determining the degree of cross-reactivity between different shellfish species. A direct amino acid sequence alignment and comparison of amino acid sequences of IgE-binding epitopes may be able to predict the level of IgE cross-reactivity. However, tropomyosin is highly conserved among various crustacean species such as prawns, crabs, and lobsters with amino acid identities reaching 95–100 %. Therefore, IgE cross-reactivity is very frequent among crustacean species (Zhang et al. 2006; Abramovitch et al. 2013; Nakano et al. 2008; Motoyama et al. 2007; Ayuso et al. 2002).

Within the mollusk group, hypersensitivity cross-reaction is often seen in allergic individuals, as determined for ten different species of cephalopods (Motoyama et al. 2006). Similar results were shown for four species of gastropods (disk abalone, turban shell, whelk, and *Middendorf's buccinum*) and seven species of bivalves

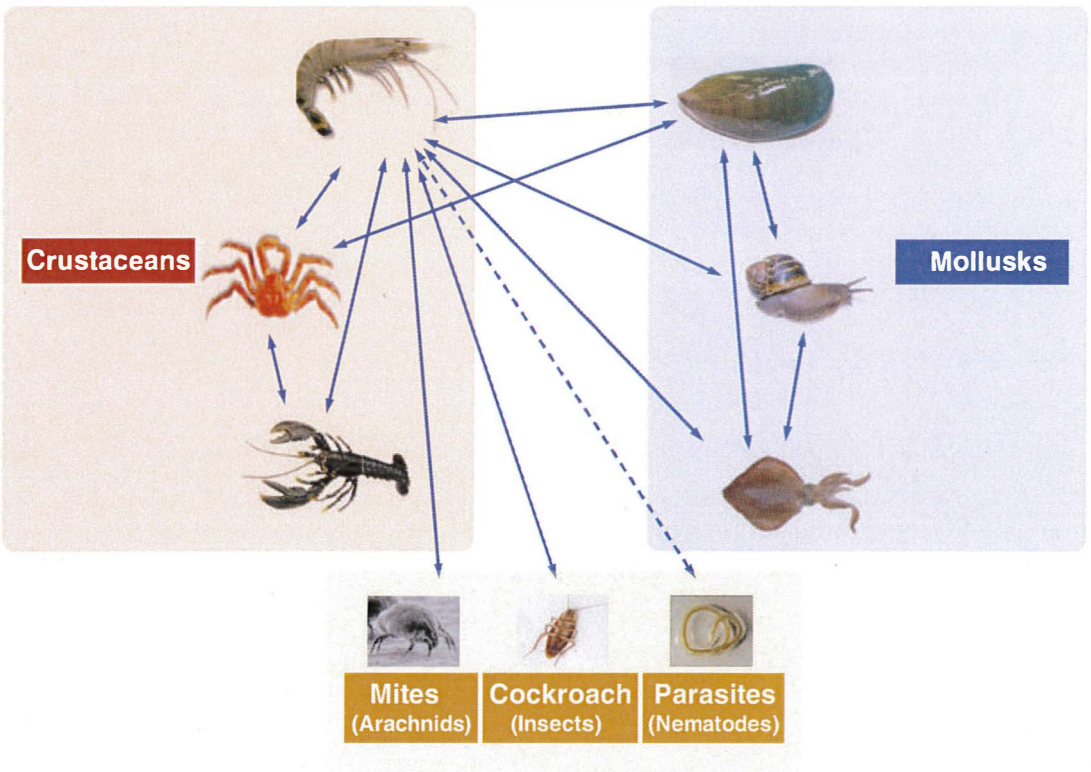

Fig. 21.2 Graphical representation of immunological cross-reactivity among crustacean and mollusk species as well as to mites, insects, and nematodes

(bloody cockle, Japanese oyster, Japanese cockle, surf clam, horse clam, razor clam, and short neck clam) (Emoto et al. 2009).

Increasingly important seems to be IgE cross-sensitization between tropomyosin from shellfish and other important allergenic invertebrates, including dust mites and cockroaches (◉ Fig. 21.2). It was demonstrated that IgE against mite tropomyosin (Der p 10) reacted very strongly to shrimp tropomyosin, although tropomyosin is present in very low concentrations in house dust mites (Arlian et al. 2009). More interestingly, reactivity to shrimp has been demonstrated in subjects with house dust mite allergy, who have never been exposed to shrimps due to religious eating habits (Fernandes et al. 2003).

21.5.1 Potential Advantages of Component-Resolved Diagnosis (CRD) in Shellfish Allergy

Applying single allergenic molecules (Matricardi et al. 2016) from shellfish for allergen-specific IgE detection could potentially modify the following:

1. Test sensitivity (improving the limit of quantitation to shellfish allergens of rare abundance or low stability)
2. Analytical specificity, particularly if specific IgE is detectable to:
   (a) Risk-associated molecules (being more likely responsible for severe reactions and/or more specific for children or adults)
   (b) Indicators of cross-reactivity (involved in broad serological cross-reactions between different shellfish species)
   (c) Markers of primary species- and/or family-specific sensitizations (facilitating the identification of unique allergic sensitizations to certain shellfish species or families)

The listed advantages of CRD require some allergen-related knowledge about the following:

- Abundance of single allergens in the shellfish body (and resulting extracts)
- Location of the allergen in the organism (edible or nonedible parts)
- Water solubility (for proper extraction)
- Stability and behavior to thermal and gastric degradation
- Frequency of sensitization to the single allergen in question
- Degree of interspecies- or interfamily-related cross-reactivity
- Risk to elicit severe allergic reactions

Specific IgE to TM, thanks to its high abundance and stability, is picked up reasonably easy using heated protein extracts from probably most shellfish species. Thus, there is no particular need to further increase test sensitivity. However, increased analytical specificity of TM in molecular-based serological tests will help to identify patients at risk for severe allergic reactions and, in addition, indicate

broad cross-reactivity to TM from other shellfish species and perhaps insects and mites. Testing IgE to more than one TM is probably providing more information about cross-reactivity between crustaceans and mollusks.

Similar assumptions are related to the other described shellfish allergens (see above), i.e., AK, MLC, SCBP, TnC, and TIM: Being part of the edible part of shellfish, with basic functions in muscle fibers or general energy metabolism, they are presumably also highly conserved, showing variable degrees of cross-reactivity, which has not been studied yet. Increasing test sensitivity through the use of single molecules might be useful in less-stable allergens (i.e., AK, TIM), but not necessarily for more robust proteins (i.e., MLC, SCBP). Increased analytical specificity can assist uncovering associated risks, i.e., in case of IgE to SCBP (Pascal et al. 2015). However, none of these candidates might serve as a single marker for species-specific sensitization due to variable degrees of IgE-related cross-reactivity, which still needs to be addressed. Recent advances in PCR-based allergen-specific IgE quantification have further improved the sensitivity and specificity of tests to single allergens, using serum from a fingerprick, which is of particular advantage for infant allergy testing (Johnston et al. 2014).

In conclusion, no species-specific allergens have been identified so far, making it difficult to precisely diagnose allergy to a specific crustacean or mollusk species with the use of allergen molecules (Matricardi et al. 2016; Aalberse 2015)). If more of the already identified and additional allergens are available for diagnostics, it might be helpful to test one per protein family, ensuring maximum test sensitivity and enhanced molecular specificity, particularly if TM is not the major allergen. This does, however, not solve the question of potential clinical cross-reactions to closely related shellfish species: Only anamnestic data or oral challenges can indicate or rule out clinically relevant allergic reactions to certain shellfish species.

21.6 Diagnostics Separating IgE-Mediated Allergy from Other Reactions

Serum-based IgE quantification tests are available for a wide variety of crustacean and mollusk species as well as for cross-reactive invertebrate species such as dust mites and cockroaches. IgE quantification tests for single-component allergens are currently only available for shrimp tropomyosin (rPen a 1). However, some additional shellfish allergens are available in multiplex (microarray) format for prawn tropomyosin (nPen m 1), arginine kinase (nPen m 2), and sarcoplasmic calcium-binding protein (rPen m 4).

Approximately 60% of patients with clinical allergy to crustacean demonstrate specific IgE binding to tropomyosin. It has been suggested that IgE reactivity to tropomyosin is a better predictor of shrimp allergy as compared to SPT or IgE to whole shrimp extract (Gámez et al. 2011; Yang et al. 2010). However, also sarcoplasmic calcium-binding protein (Pen m 4) reactivity has been associated with clinical reactivity to shrimp. The combination of reactivity to both allergens might increase the sensitivity to detect clinically allergic patients, but has still to be confirmed.

The consumption of seafood is very different from most other food allergen sources. It can trigger clinical adverse symptoms, although nonallergic in origin, being similar in clinical presentation to true IgE-mediated allergic reactions. These substances are found in seafood much more frequently as compared to any other food source. An atypical clinical history or an inconsistent history always suggests a nonatopic etiology, such as contamination with marine biotoxins, parasites, bacteria, and viruses (Lopata et al. 2010; Lopata and Kamath 2012). Because of the similarity in clinical reactions of affected individuals, it is essential to differentiate adverse reactions from true shellfish allergy and understand the molecular nature of the offending allergens for improved component-resolved diagnosis.

Food challenge or double-blind placebo-controlled food challenge (DBPCFC) can be performed to confirm clinical reactivity to crustacean and mollusk species. However, such provocation tests are not performed routinely because of increased risk and costs and are only performed for investigating individual cases.

21.7 Outlook for Future Diagnostic Options

Most of the clinical studies on cross-reactivity have been conducted using tropomyosin as the major pan-allergen. However, other shellfish allergens may play a role in immunological cross-sensitization. A recent study has shown that allergens other than tropomyosin, such as arginine kinase, might also be responsible for cross-reactivity between shellfish and inhalant invertebrate allergen sources (Gamez et al. 2014; Marinho et al. 2006). In addition, hemocyanin has been demonstrated to be cross-reactive and also is a known cockroach allergen (Giuffrida et al. 2014; Khurana et al. 2014).

However, an in-depth investigation into the conservation or relevance of specific IgE epitopes between pan-allergens from crustaceans and mollusks and clinical cross-reactivity to mites and cockroaches have not been conducted or confirmed using a larger number of shellfish-allergic patients.

21.8 Suggestions for Present Clinical Practice

Diagnosis of shellfish allergy is based on:

- Clinical history
- Sensitization tests (allergen-specific IgE tests; skin tests)
- Oral challenge test, if needed

In case of severe allergic reaction, allergen-specific IgE should precede any in vivo tests, i.e., skin prick test (SPT), to avoid any risks for the shellfish-allergic patient.

IgE diagnostics should include:

- Total IgE (for improved interpretation of the quantitative allergen-specific IgE values)

- Allergen-specific IgE preferably to the reaction-eliciting (or biologically closely related) shellfish species
- Allergen-specific IgE to Pen a 1, at the present only available TM for singleplex testing from brown shrimp (*Penaeus aztecus*):
  A. If extract- and TM-specific IgE results are positive with quantitative IgE levels being higher to TM than to the whole extract, immunodominant sensitization to shellfish TM is likely, and broad (serological) cross-reactivity to other shellfish species is to be expected. During interpretation of the test, concordance between recorded symptoms and the identified shellfish species should be checked. Only in case of corresponding symptoms and a positive sensitization test, clinically relevant allergy has successfully been demonstrated.
  B. If only the extract-specific IgE, but not the TM-specific IgE is positive, sensitization to TM is unlikely, but other shellfish allergens might be involved.
  C. If both IgE tests (shellfish extract- and TM-specific IgE) turn out to be negative, it is mandatory to perform a skin test, i.e., SPT with a commercial shellfish extract and/or a (titrated) SPT with native material (i.e., prick-prick test with fresh shellfish species, if possible raw and cooked).
  D. In case of a clearly positive SPT result, an immediate-type sensitization is likely, particularly if healthy control individuals do not react to the applied skin test material.
  E. In case of clearly negative skin test results, IgE-mediated sensitization to the tested shellfish species becomes very unlikely, and differential diagnoses other than IgE-mediated allergic reactions to shellfish should be considered.
  F. Additional testing with other shellfish species has limited value for subsequent consulting of the patient: In case of positive skin or IgE test results, serological cross-reactivity has been demonstrated, which does not always translate into clinical cross-reactivity. However, in case of a clearly negative skin and/or IgE response to related or biologically more distant shellfish specifies (serological), cross-reactivity and subsequent clinical cross-reactivity becomes unlikely.
  G. In case of doubt or mismatch between case history and diagnostic results, carefully titrated oral challenge tests with the suspected shellfish species might solve the discrepancies. However, due to the risk for the patient in case of previous severe allergic reactions and limited specialized centers, they are not frequently performed. A negative provocation test, if previous sensitizations tests turned out negative, is usually safe and an appropriate way to rule out a present food allergy to shellfish.

In general, patients with proven shellfish allergy should avoid a broad range of related shellfish species (crustacean or mollusk), unless they have already tolerated other (presumably biologically more distant) shellfish species. This rather cautious approach takes into account that allergic subjects are not necessarily familiar with huge variety of present shellfish species, their biological relationship, and the composition in mixed seafood dishes, particularly from nonself-prepared meals.

Due to the often long-lasting nature of IgE-mediated allergies to shellfish species, patients with proven allergic reactions should avoid shellfish permanently, unless subsequent controlled challenges have ruled out a still-present clinical reactivity.

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