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Shellfish Allergy: a Comprehensive Review

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Abstract Shellfish allergy is of increasing concern, as its prevalence has risen in recent years. Many advances have been made in allergen characterization. B cell epitopes in the major allergen tropomyosin have been characterized. In addition to tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, and myosin light chain have recently been reported in shellfish. All are proteins that play a role in muscular contraction. Additional allergens such as hemocyanin have also been described. The effect of processing methods on these allergens has been studied, revealing thermal stability and resistance to peptic digestion in some cases. Modifications after Maillard reactions have also been addressed, although in some cases with conflicting results. In recent years, new hypoallergenic molecules have been developed, which constitute a new therapeutic approach to allergic disorders. A recombinant hypoallergenic tropomyosin has been developed, which opens a new avenue in the treatment of shellfish allergy. Cross-reactivity with species that are not closely related is common in shellfish-allergic patients, as many of shellfish allergens are widely distributed panallergens in invertebrates. Cross-reactivity with house dust mites is well known, but other species can also be involved in this phenomenon.

Keywords Arginine kinase · Myosin light chain · Sarcoplasmic calcium-binding protein · Shellfish allergy · Tropomyosin

Shellfish are a widely consumed source of protein, given their low caloric content and high nutritive value. Shellfish belong to the subkingdom *Eumetazoa*. There are more than 50,000 crustacean species and 100,000 mollusk species. The most frequently consumed species are summarized in Table 1.

According to the Food and Agriculture Organization (FAO), global shellfish consumption in 2009 was 1.7 kg person^{-1} year^{-1} of crustaceans, 2.4 kg person^{-1} year^{-1} of mollusks and 0.5 kg person^{-1} year^{-1} of cephalopods (faostat.fao.org).

Epidemiology

Data on the prevalence of shellfish allergy are limited due to the lack of controlled population studies in which an oral food challenge is performed. A recent review states that the prevalence of shellfish allergy in children is less than 0.5 % [1]. In 2004, a telephone survey of 14,948 participants conducted in the USA revealed that 2 % reported allergy to any seafood [2]. In a study of 227 Singapore children with food allergy, sensitization to crustaceans was found in 39 % [3] and in Spain 6.8 % of 355 children had positive skin tests to crustaceans [4].

Epidemiologic studies in Spain show that shellfish allergy has significantly increased and is the third leading cause of allergic reactions to foods [5]. Eighty-five percent of the reactions were caused by crustaceans, which is consistent with data published in Australia [6] where crustaceans are involved in 87 % of the reactions.

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Table 1 Taxonomic classification of the most frequently consumed shellfish

Subkingdom	Phylum	Subphylum	Class	Subclass	Infraclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Common name
Eumetazoa	Mollusca		Bivalvia	Heterodonta			Veneroida			Veneridae	Ruditapes Tapes	Clam
										Cardiidae	Cerastoderma	Cockle
				Pteriomorphia			Mytiloida			Mytilidae	Mytilus	Mussel
							Ostreoida			Osteidae	Ostrea	Oyster
										Pectinidae	Pecten	Scallop
			Cephalopoda	Coleoidea		Octopodiformes	Octopoda			Octopodidae	Octopus	Octopus
						Decapodiformes	Sepiidae			Sepia	Sepia	Sepia
							Teuthida			Ommastephidae	Todarodes	Squid
										Loliginidae	Loligo	Squid
			Gasteropoda	Orthogastropoda			Pulmonata			Hellixidae	Helix	Snail
				Patellogastropoda						Patellidae	Patella	Limpet
							Archaeogastropoda			Haliotidea	Haliotis	Abalone
	Arthropoda	Crustacea	Malacostraca	Eumalacostraca		Eucarida	Decapoda	Dendrobranchiata		Penaeoidea	Penaeus	Shrimp
											Litopenaeus	Shrimp
											Parapenaeus	Prawn
								Pleocyemata	Caridea	Palaemonoidea		Shrimp
									Astacidea	Astacoidea Parastacoidea		Crayfish
									Palinura	Nephropoidea	Nephrops	Lobster
										Palinuridae	Hommarus	American/European lobster
											Palinurus	Spiny lobster
									Brachyura	Cancridae	Cancer	Crab
										Majidae	Maja	Spider crab
										Portunidae	Necora	Velvet crab
			Maxillopoda	Thecosarca	Cirripedia		Pedunculate			Scalpellidae	Pollicipes	Barnacle

Seafood allergy is more common in adults; however, in schoolchildren the prevalence is around 5 % [5].

Allergens

Tropomyosin (TM) was the first allergen identified in shellfish, but other allergens have recently been characterized: arginine kinase (AK), myosin light chain (MLC), and a calcium-binding sarcoplasmic protein (SCP), among others. Table 2 summarizes the shellfish allergens described to date.

Tropomyosin

TM was first described as a crustacean allergen in shrimp in 1981 [44]. In 1993, Shanti et al. [45] noted the presence of TM as a soluble allergen in the heat-stable fraction that dominated the allergenicity of the extract. TM consists of 281 amino acids and has a molecular weight (MW) of 38–41 kDa, (Fig. 1a).

TMs are present in both muscle and nonmuscle cells. In striated muscle, they mediate the interaction of the troponin (Tn)–actin complex to regulate muscle contraction. TM is a coiled-coil protein formed by two parallel α-helices containing two sets of seven alternating binding sites on actin (heptads) (Fig. 2). The Tn–TM complex regulates the Ca^{2+}-dependent interaction of actin and myosin. The TM molecule forms a continuous chain along the actin filament by "head to tail" polymerization. Each TM molecule binds seven actin monomers through Tns I, T, and C (the calcium-binding subunit).

In nonmuscle cells, many isoforms have been purified in bovine thyroid, porcine kidney, rabbit lung macrophages, human erythrocytes, porcine platelets, chicken embryo fibroblasts, and *Drosophila* embryo, where they are involved in mRNA location [46]. It is believed that the role of TM in nonmuscle cells is to provide mechanical support to the cytoplasmic membrane and to the transport of other molecules.

In 1989, the thermostable allergen was isolated for the first time in shrimp (*Penaeus indicus*) and was designated as Sa-II [47]; it was later renamed according to the allergen standard nomenclature as Pen i 1. Other TMs were subsequently identified in various shrimp, lobster, and crab species [7–12] and as a major allergen in mollusks: snails [13], abalone [14–16], whelk [17], horned turban [18], clam [19], razor shell [20], mussels [16, 21], oysters [22], scallops [16], octopus [23], and squid [24–26].

Invertebrates TM are panallergens, whereas vertebrate TMs appear to be nonallergenic [48]. With the aid of bioinformatic methods that compare TM sequences, it has been determined that vertebrate TM (rabbit, chicken, pig, and human) share 53–57 % sequence identity with shrimp TM (Met

Table 2 Allergens identified in shellfish

Name	MW	Function	Sources	Examples	References
Tropomyosin	38–41 kDa	Muscle contraction	Shrimp, lobster, crab, snail, abalone, whelk, clam, mussels, and octopus	Pen a 1 Pen m 1 Hal d 1 Oct f 1	[7–26]
Arginine kinase	40 kDa	Metabolic role (regulation and transport)	Shrimp, crab, and octopus	Pen m 2 Lit v 2	[27–33]
Myosin light chain	20 kDa	Muscle contraction	Shrimp and lobster	Lit v 3 Cra c 5 Hom a 3	[34–36]
SCP	20–22 kDa	Muscle contraction	Shrimp	Pen m 4 Lit v 4	[37–39]
Hemocyanin	75 kDa	Oxygen transport	Shrimp	Mac ro 2	[40]
Troponin C	21 kDa	Muscle contraction	Shrimp and lobster	Cra c 6 Hom a 6	[35, 41]
Paramyosin	100 kDa	Muscle contraction	Abalone, turban shell, mussel, and octopus		[42]
Triose phosphate isomerase	28 kDa	Glycolytic enzyme	Shrimp	Cra c 8	[35]
Myosin heavy chain	225 kDa	Muscle contraction	Shrimp and snail		[41, 43]
α-actin	31–42 kDa	Muscle contraction	Shrimp		[28, 41]
SERCA	113 kDa	Enzyme	Crab		[28]
GADPH	37 kDa	Enzyme	Shrimp		[41]

MW molecular weight, *SCP* sarcoplasmic calcium-binding protein, *SERCA* smooth endoplasmic reticulum Ca^{2+} ATPase, *GADPH* glyceraldehyde phosphate dehydrogenase

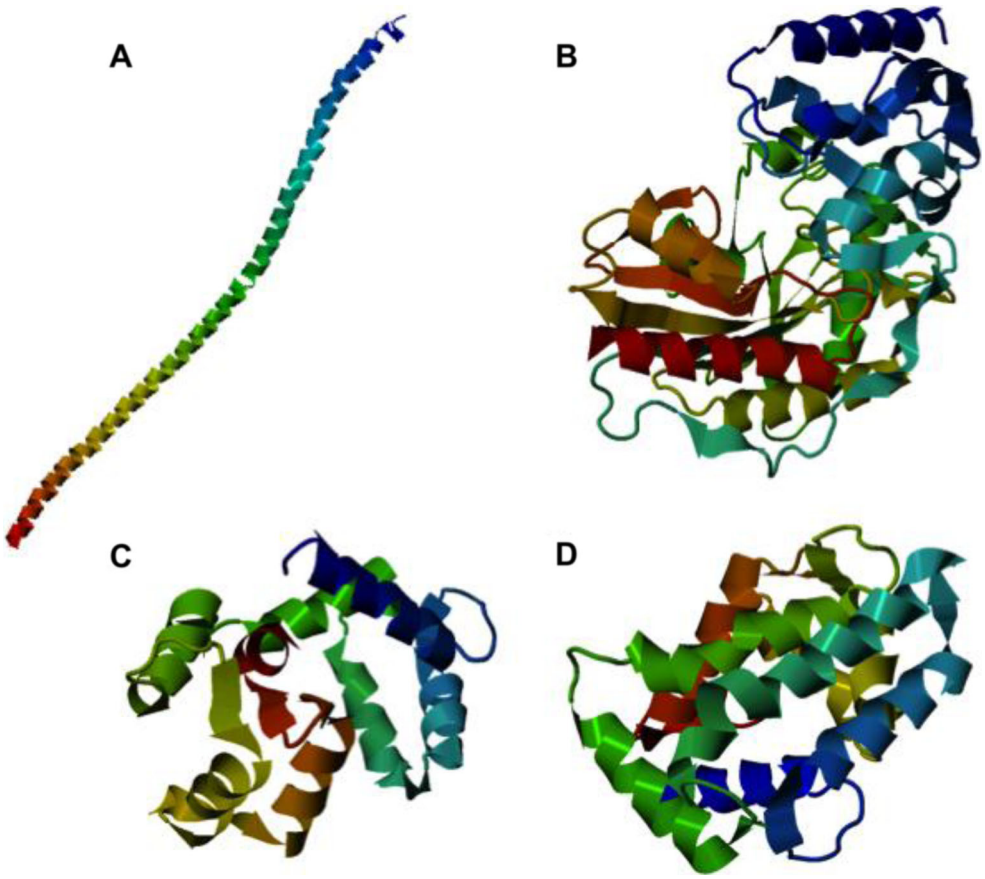

Fig. 1 Shellfish allergens: **a** Tropomyosin (PDB accession number A1KYZ2). **b** Arginine kinase (PDB accession number Q004B5). **c** Myosin light chain (PDB accession number P08052). **d** Sarcoplasmic calcium-binding protein (PDB accession number P02637)

e 1) [49]. This homology could explain why the vertebrate TMs are nonallergenic and do not show cross-reactivity with the TMs of invertebrates. However, the amino acid sequence identity between mollusk TMs varies from 68 to 88 % and between crustaceans and mollusks is 56–68 %, which is slightly higher than that with vertebrate TMs. This suggests that TM is not solely responsible for the cross-reactivity of shellfish.

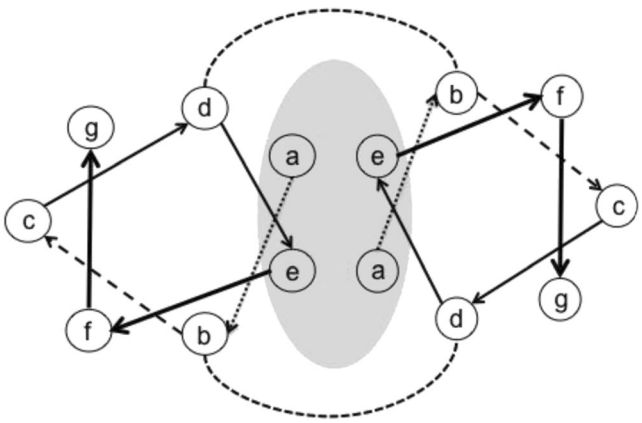

Fig. 2 Representation of two repetitive heptads from two α-helices, which shows how coiled-coil structures are formed

TM is a stable allergen that resists heat and typical food processing treatments. Many studies have addressed the effects of various types of processing on TM (Table 3). The change in allergenicity of Japanese scallops (*Patiopecten yessoensis*) after Maillard reactions was studied [50]. In this study, an increase in IgE-binding ability was found for TM in the early stages of the reaction with glucose, ribose and maltose, but not with maltotriose. The treatment of TM lysine residues with 2,4,6-trinitrobencensulfonic acid resulted in no effect on its allergenicity. Thus, it was concluded that the loss of positive charges on the surface of the protein is not responsible for the increased allergenicity, but rather that the increased allergenicity is related to the structural change resulting from nonenzymatic glycosylation. Conversely, IgE-binding ability was reduced as the reaction progressed and persisted despite peptic digestion in the case of squid TM (*Todarodes pacificus*) [51]. These differences can be explained by fact that the homology between squid and scallop TM is only 69.7 %, and the presence of different epitopes is presumed.

Ultrasound (US) on shrimp TM [52] decreases IgE-binding ability depending on the length of treatment, reaching 25 % (measured by enzyme-linked immunosorbent assay (ELISA) with polyclonal antibodies) after 180-min 30 Hz, 800 W. The effect of various processing methods on the allergenicity of

Table 3 Effects of physical or chemical treatments on TM allergenicity

Source	Treatment	Effect on IgE binding	Reference
Scallop (*Patiopecten yessoensis*)	Maillard reaction	Increase	[50]
Squid (*Todarodes pacificus*)	Maillard reaction	Decrease	[51]
Shrimp (*Penaeus vannamei*)	US (180 min, 30 Hz, 800 W)	Decrease	[52]
Shrimp (*Scylla paramamosain*)	CUB/HPS	Decrease	[53]
Shrimp (*Penaeus monodon*, *Litopenaeus vannamei*)	Enzymatic digestion	Decrease	[54]
Shrimp (*Penaeus japonicus*)	Heating–recooling	Decrease recovering	[55]
Shrimp (*Litopenaeus setiferus*)	Pulses of UVL	Decrease	[56]

US ultrasound, *CUB/HPS* combined ultrasound and boiling/high-pressure steaming, *UVL* ultraviolet light

mud crab TM (*Scylla paramamosain*) has been investigated [53]. The digestive stability of TM treated by boiling, combined US and boiling (CUB), and high-pressure steaming (HPS) has been investigated. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and ELISA inhibition assays revealed that boiling has a limited effect on the digestive stability of TM, but CUB or HPS diminishes IgE-binding ability. The most effective method for reducing the allergenicity of TM was HPS. Treatment with US and HPS leads to changes in the TM structure, weakening bond interactions, and facilitating protease degradation.

Recently, the effect of enzymatic digestion (pepsin, trypsin, and chymotrypsin) on shrimp TM has also been investigated [54]. After 4 h of enzymatic treatment, the protein is partially digested and its IgE-binding ability is decreased. Nevertheless, later studies with circular dichroism (CD) [55] have shown that although shrimp TM loses its α-helix structure after heating to 80 °C, there is no evidence of aggregate formation. The helix structure is recovered when TM is cooled to 25 °C thus maintaining its antigenicity.

Currently, the food processing industry uses ultraviolet light pulses for microbe inactivation. It has been shown that these pulses reduce the allergenicity of shrimp TM (*Litopenaeus setiferus*) in crude and boiled extracts, and this reduction is proportional to the duration of the pulses [56].

To date, some B cell epitopes have been described in TM. In 1993, Shanti et al. [45] described two peptides in shrimp TM (residues 50–66 and 153–161). Later, Ayuso et al. [57, 58], using overlapping peptides, described five major IgE-binding regions and 22 minor peptides in shrimp TM. All had a length between 15 and 38 amino acids (region 1, 43–57; region 2, 85–105; region 3a and b, 133–148; region 4, 187–202; and region 5a–c, 247–284). Region 1 was recognized by 55.5 % of the sera, region 2 by 83.3 %, region 3 by 55.5 %, region 4 by 27.5 %, and region 5 by 66.6 %. These regions are positioned in the molecule at regular intervals of 42 amino acids (seven heptads), which suggests a relation with the coiled-coil structure. The amino acid sequences in these five regions were compared with their correspondents in other vertebrate and invertebrate TMs. Region 1 was identical in all crustaceans; regions 2 and 4 showed 100 % homology with that of cockroach, fruit fly, and dust mites and up to 85 % with those of some vertebrates. Regions 3 and 5 were identical among crustaceans and showed 89 % homology with those of arthropods; however, they differed from those of vertebrates. The analysis of these epitopes and their homology with the same regions of other allergenic sources explains, at least in part, the cross-reactivity among different species.

Another approach based on informatics has been used for the study of shrimp TM epitopes [59]. Briefly, three separate computer systems predicted potential epitopes, based on a combination of properties of the amino acids (hydrophobicity, flexibility, accessibility, loops, exposed surfaces, polarity, and antigenic propensity properties). The potential epitopes for which there was agreement between at least two of the computer systems were synthesized and validated by means of inhibition assays. With this computer-based approach, ten candidate peptides were obtained (peptide 1, 23–40; peptide 2, 45–59; peptide 3, 89–105; peptide 4, 115–128; peptide 5, 131–142; peptide 6, 145–164; peptide 7, 177–190; peptide 8, 210–224; peptide 9, 243–259; and peptide 10, 263–280). Seven of them totally or partially contained those previously described by Ayuso et al. [57]. Eight of the ten peptides reacted with more than half of the sera. This approach is interesting, as it is easier and cheaper than the synthesis of overlapping peptides.

Arginine Kinase

In 2003, Yu et al. [27] characterized a new shrimp allergen, Pen m 2 (MW=40 kDa), by means of two-dimensional immunoblotting and MALDI-TOF. Pen m 2 was recently determined to have AK activity. This allergen was recognized by 94 % of patients. Snowcrab (*Chionoecetes opilio*) AK was shown to be aerosolized in air samples of crustacean processing factories and was recognized by 43 % of patients [28]. AK has also been described in other crustaceans [29–33] and mollusks [31], showing high sequence identity.

Thermal processing and acid-base treatment has shown to reduce the allergenicity of this protein.

AK (Fig. 2b) is, like the majority of enzymes, a thermolabile allergen. It has regulation and transport properties and is abundant in crustacean muscle. AK is a phosphagen kinase that plays a crucial role in invertebrate's metabolism, catalyzing the reversible transference of a high-energy phosphate from arginine phosphate to ADP to form ATP [28]. Molecular analyses of crustacean AK have revealed an evolutive relationship with creatinine kinase from vertebrates, which plays a similar metabolic role.

As with TM, AK is an invertebrate panallergen, as it has been described not only in crustaceans and mollusks but also in moths [60], mites [61], silkworms [62], spiders [63], fruit flies [64], and cockroaches [65, 66].

Myosin Light Chain

In 2008, Ayuso et al. [34] described 177 new amino acids, 20 kDa, and p*I* 4.2 allergen in shrimp (*Litopenaeus vannamei*) named Lit v 3, which was identified as a MLC (Fig. 2c). It shows 66 % sequence homology and 51 % identity with MLC from cockroaches (Bla g 8). MLC was recognized by more than 50 % of the studied patients in both raw and boiled extracts. IgE binding to boiled extract was higher in adults than children, who tended to recognize MLC in the raw extract more intensely. Some of these children had symptoms upon exposure to boiling vapors, so the authors suggested that MLC might be aerosolized and contribute to the development of these symptoms. Along these lines, a previous study [67] described a case of occupational asthma in a seafood restaurant worker whose IgE recognized a 21- to 26-kDa protein in raw shrimp extract and in the boiling water, which could correspond to MLC. Recently, MLC has also been described in North Sea shrimp [35] and in lobster [36].

Myosins are a large superfamily of muscular proteins that take part in muscle contraction, moving along actin filaments as they hydrolyze ADP. Myosin is formed by two heavy chains, wrapped in a 20-kDa light chain. In a relaxed state, Tn is bound to myosin to prevent it from binding actin. During muscular contraction, Ca^{2+} levels rise and binds Tn C, which changes its structure, making TM release myosin and liberating the binding sites of actin. The actin–myosin binding complex is influenced by MLC phosphorylation that produces conformational changes in myosin, allowing muscular contraction.

Sarcoplasmic Calcium-binding Protein

In 2008, Shiomi et al. [37] described a 20-kDa allergen in shrimp (*Penaeus monodon*), which was identified as a sarcoplasmic calcium-binding protein (SCP) (Fig. 2d) recognized by 50 % of studied patients.

This finding was later confirmed by Ayuso et al. [38] in *L. vannamei* (Lit v 4.0101, 199 amino acids; MW, 22 kDa; calculated p*I*, 4.7). Recombinant SCP (rSCP) was recognized by 38 % of the patients. This percentage was particularly high in children (74 vs. 10 % in adults). Inhibition experiments with lobster and crab suggest the presence of cross-reactive epitopes in SCP. Nevertheless, inhibition with mite extract, cockroaches, or mollusks was not significant. Mita et al. [39] showed a high percentage of sequence homology between crustacean SCPs that varied between 80 and 98 %, although between crustaceans and mollusks it is only 15–21 %, suggesting that SCP is involved only in cases of cross-reactivity between crustaceans.

SCPs are acidic, calcium-binding *EF-hand* proteins present in cytosol, with a MW of 20–22 kDa. Shrimp SCP are dimmers of polypeptide chains (αα, αβ, and ββ) with three calcium-binding sites [68]. SCP is believed to play a similar role as parvalbumin, which is to promote muscular relaxation by translocating Ca^{2+} from myofibrils to the sarcoplasmic reticulum. SCP is a glycoproteic allergen [69] containing, as determined by the phenol-sulfuric method, 4.9 % carbohydrates. It is thermostable and resistant to treatment with acid and alkalis (stable in a buffer with pH range of 1–11). However, after 1 h of peptic digestion, it is completely digested. SCP is a polymorphic allergen with three isoforms (SCP-I, SCP-II, and SCP-III) [68] with p*I* 5.05, 4.90, and 4.75, respectively, all of which show IgE-binding capacity [69].

Hemocyanin

Hemocyanin has been described [40] in freshwater shrimp (*Macrobrachium rosenbergii*). The authors studied patients allergic to *M. ronsenbergii*, who tolerated seawater shrimp (*P. monodon*), as confirmed by an oral food challenge. *M. ronsenbergii* SDS-PAGE showed prominent bands at MW 40, 50, and 70 kDa. Proteins were isolated by anion exchange chromatography and later analyzed using LC-MS/MS. Peptides obtained from 72 and 75 kDa bands were characterized as hemocyanin and showed 62.5–100 % sequence homology with other crustaceans; nevertheless, homology with *P. monodon* was as low as 18.8–27.3 %. These findings are in accordance with those of the inhibition experiments, in which *P. monodon* failed to inhibit *M. rosenbergii*. This study also revealed the stability of the protein after thermal processing, as binding to IgE was detected in both raw and boiled hemolymph extracts.

Previously, Juji et al. [70] described the case of a young woman who presented two episodes of food-induced, exercise-dependent anaphylaxis after ingestion of limpet and horned turban. Both extracts showed cross-reactivity in RAST-inhibition studies and with keyhole limpet.

Hemocyanin is an oxygen-transport protein [71, 72] found in crustacean hemolymph, which accounts for 75–95 % of the

total protein content. Hemocyanin in its natural state forms hexamers or multihexamers of individual subunits of 75 kDa. Different subunits are species specific [71].

Troponin C

Troponin C was first described as an allergen in North Sea shrimp (*Crangon crangon*, Cra c 6) [35], recognized by 29 % of the patients and later in *Pandalus borealis* [41], recognized by 33 %.

Tn is formed from three subunits: Tn C, which binds to Ca^{2+}, Tn I, which binds to actin and inhibits actin–myosin interaction, and Tn T, which binds to TM [73].

Tn C belongs to a family of homologous proteins that includes calmodulin, MLC, and parvalbumins. Sequence analysis has revealed four calcium-binding regions (I–IV), all of which contain a pair of helices flanking the 12-residue calcium-binding site. Crystallographic studies have shown two independent domains connected by a central helix, containing two calcium-binding sites each. There are two high-affinity sites (III and IV) and two low-affinity sites (I and II) that are responsible for muscular contraction. However, only regions II and IV are functional [74].

Paramyosin

Paramyosin has recently been recognized as a 100-kDa thermolabile allergen in various mollusk species [42], recognized by 16 out of 18 serum samples, and it has shown to be cross-reactive with TM.

Paramyosin is an invertebrate-specific protein that forms the core of filaments that contain myosin.

Triose Phosphate Isomerase

Triose phosphate isomerase (TIM) is a minor allergen in crustaceans (Cra c 8) [35], recognized by 23 % of the patients. Other members of this family have been described as allergens in fish [75].

Myosin Heavy Chain

In 2005, Martins et al. [76] studied patients allergic to snails (*Helix aspersa*) and described two bands with MW higher than 208 kDa in SDS-PAGE that were recognized by immunoblotting and were believed to correspond to myosin heavy chain (MHC; MW, ~225 kDa). These allergens were recognized by 13 and 18 of 21 patients, respectively. In the study, these bands were also recognized in the extracts of *Theba pisana* and *Otala lacteal*, so the authors suggested that MHC might be involved in cross-reactivity between mollusks, crustaceans and arachnids.

More recently [41], MHC was also recognized by 11 % of the shrimp-allergic patients (workers in a shrimp processing factory).

Other Allergens

Other minor allergens have been described, such as α-actin [28, 41] with a MW 31–42 kDa, which was recognized by 22 % of the patients [41]; smooth endoplasmic reticulum Ca^{2+} ATPase (SERCA) with MW 113 kDa [28], and glyceraldehyde phosphate dehydrogenase (GADPH) with MW 37 kDa, which was recognized by 44 % [41].

Clinical Symptoms

Adverse reactions to shellfish can be mediated by immunologic or non-immunologic mechanisms as a result of exposure to shellfish itself or to other components of the ingested product. Reactions can be triggered by many substances, such as as parasites (*Anisakis simplex*), protochordates (Hoya), bacteria (*Vibrio*, *Klebsiella*, and *Pseudomona*), viruses (hepatitis A virus), toxins (saxitoxins and ciguatera), or biogenic amines, preservatives, flavorings, and colorings (sodium benzoate or metabisulfites) [77].

Some studies suggest that shellfish are one of the most frequent causes of allergic reactions to foods, and that shellfish cause more severe reactions compared with other foods [78], including reactions such as anaphylaxis, which lead to the need for emergency care [79, 80].

Symptoms developed after the ingestion of shellfish are similar to those presented with other foods. Reactions are immediate and occur a few minutes after ingestion, almost always within the first 2 h. Nevertheless, some cases of late reactions occurring 8 h after the ingestion of snow crab, cuttlefish, limpet, and abalone, have been reported [15, 81]. Food-induced, exercise-dependent anaphylaxis after the ingestion of oysters [82], squid [83], and scallops [84] have also been documented.

The clinical presentation of shellfish allergy includes cutaneous symptoms (82 %), oral allergy syndrome (OAS; 28 %), digestive symptoms (18 %), anaphylaxis (20 %), asthma (5 %), and rhinitis or exercise-dependent asthma (<5 %) according to Spanish data [5]. A recent study in Australia [6] showed that patients suffered anaphylaxis in 21 % of cases and 15 % presented contact urticaria. In China [85], 63 % of shellfish-allergic patients reported isolated cutaneous symptoms, 33 % had suffered anaphylaxis and 2.4 % had asthma. In this study, no association was found between developing anaphylaxis and a previous diagnosis of asthma, nor with the involved shellfish, with the exception of abalone and limpet, in which anaphylaxis was more frequent. A later study

[86] with oral food challenge (OFC) showed that mucocutaneous symptoms were more frequent (95.7 %), followed by respiratory (23.9 %), gastrointestinal (16.3 %), anaphylaxis (11.9 %), and cardiovascular (3.3 %) symptoms.

Allergic symptoms can be triggered not only after ingestion but also after exposure to vapors in occupational or home settings [67, 87, 88]. This exposure route triggers respiratory, cutaneous, and rarely, systemic reactions.

Diagnosis

Diagnosis is based on the demonstration of the presence of specific IgE (sIgE) and includes skin prick tests (SPT), and/or quantification of sIgE by means of ImmunoCAP or allergen microarrays. However, a positive result is not proof of clinical reactivity, thus diagnosis must be based on a clinical history. The clinical record must include aspects such as the offending food, symptoms, the lapse between the intake and the development of symptoms, the required treatment, the duration of symptoms, the number of reactions and possible triggering factors such as NSAIDs or exercise. This information helps the clinician to distinguish between immediate hypersensitivity reactions and reactions triggered by parasites or other substances. Aspects such as the development of symptoms upon contact, exposure to vapors and tolerance to other species after the reaction should also be recorded as this will improve the preventive measures that should be offered in each case.

SPT is a safe and rapid method for screening patients with suspected allergy to shellfish. A study in Thailand [86] that included 68 patients who underwent OFC aimed to compare different SPT extracts (in-house vs. commercial) and prick–prick with *P. monodon* and *M. rosenbergii* (Table 4). Prick–prick showed the best diagnostic performance, thus this technique is recommended for those patients with a suggestive history of allergy to crustaceans [89]. A cutoff point in SPT was set at 30 mm for the in-house extract of *P. monodon*, which provided 80 % predictive probability for a positive challenge and 20 mm for commercial extract and 22.5 mm for prick–prick, which provided 95 % predictive probability [86].

A previous study [90] showed that proteins contained in raw and boiled extracts of shrimp and two lobster species were slightly different. SPTs were performed with raw and boiled extracts of the three species on 78 patients. Boiled extracts showed better diagnostic performance, as they detected 4 % more patients who were sensitized to shrimp, 18 % more who were sensitized to American lobster, and 19 % more who were sensitized to spiny lobster than raw extracts did.

A previous study in China showed that 16.7 % of the patients who reported an allergic reaction after ingestion of shellfish had a negative SPT, and half of them suffered anaphylaxis. On the other hand, 65 % of the patients presented more than one positive SPT and the mean number of positive SPT to any shellfish per patient was 2.61.

In vitro diagnostic methods include the determination of sIgE. Currently, there are 14 shellfish and one shrimp TM (rPen a 1) available by ImmunoCAP (ThermoFisher). Detection of sIgE to shrimp does not always correlate with clinical symptoms, as there are atopic patients who may have false positive results. Atopic patients usually have higher IgE levels than nonatopic patients [91], so the results of the studies are not comparable in most cases. Nevertheless, no cutoff point for sIgE to shellfish has been defined, and there is still the problem of in vitro cross-reactivity without clinical expression.

A study of mite-allergic patients who were examined for shellfish allergy has been recently published [92]. Thirty-five patients were selected, 20 of whom were sensitized to shrimp by means of SPT or serum sIgE. An OFC with shrimp was performed, with a positive outcome in six cases. OFC was also performed on nonsensitized patients, and one positive result was obtained. Serum sIgE against TM was determined and resulted positive in seven patients, five of whom had a positive OFC. Data on the diagnostic performance of SPT and sIgE are shown in Table 5. The determination of sIgE against TM was found to be the most specific and had a higher PPV. In line with these results, Gamez et al. [93] showed that the determination of sIgE to rPen a 1 (ImmunoCAP) has a PPV 0.72 and a negative predictive value (NPV) of 0.91 in a population of 45 patients with suspected shrimp allergy, 18 of whom had confirmation of diagnosis by OFC.

Recently, Ayuso et al. [94] investigated the response of IgE to overlapping peptides in shrimp allergens (*L. vannamei*): Lit

Table 4 Diagnostic performance of skin prick tests with commercial extract, in-house and prick–prick with two shrimp species

	Sensitivity	Specificity	PPV	NPV
Commercial extract	88.3 %	37.5 %	91.4 %	30 %
Prick–prick *Penaeus monodon*	100 %	41.7 %	65.7 %	100 %
Prick–prick *Macrobrachium rosenbergii*	100 %	0 %	70.6 %	NA

Modified from Jirapongsananuruk et al. [86]

PPV positive predictive value, *NPV* negative predictive value, *NA* not applicable

Table 5 Diagnostic performance of skin prick tests, shrimp, and tropomyosin sIgE in patients allergic to mites

	Sensitivity	Specificity	PPV	NPV	Efficiency
Prick test	71.4 %	64.2 %	33.3 %	90 %	65.7 %
Shrimp sIgE	71.4 %	75 %	41.6 %	91.3 %	74.2 %
TM sIgE	71.4 %	92.8 %	71.4 %	92.8 %	88.5 %

Modified from Yang et al. [92]

PPV positive predictive value, *NPV* negative predictive value, *TM* tropomyosin

v 1 (TM), Lit v 2 (AK), Lit v 3 (MLC), and Lit v 4 (SCP), using microarray technology and compared it with the response of IgE to natural shrimp allergens by means of immunoblotting. Patients with a positive OFC showed greater epitope recognition to the four allergens, both in number and intensity. In particular, some epitopes in Lit v 1 and Lit v 2 were much more efficient, suggesting that these epitopes may be used as biomarkers of clinical reactivity in sensitized subjects. However, these results need to be validated in larger groups of clinically well-characterized patients.

A panel of North Sea shrimp allergens containing Cra c 1 (TM), Cra c 2 (AK), Cra c 4 (SCP), Cra c 5 (MLC), Cra c 6 (TnC), and Cra c 8 (TIM) has been developed [35]. Allergens were recognized by 68, 29, 35, 19, 29, and 23 % of the patients, respectively. Ninety percent of the patients recognized at least one allergen. However, the panel did not show better sensitivity than shrimp extract, which was positive in 97 % of the cases.

The gold standard for food allergy diagnosis is a double-blind, placebo-controlled food challenge (DBPCFC). It is the first choice when subjective symptoms are present [95, 96]. Patients who have suffered an episode of anaphylaxis or who have poorly controlled asthma should be excluded from this challenge [95].

The dose-eliciting symptoms varies depending on the study. Wu and Williams [85] reported a fatal case of anaphylaxis after the ingestion of three snails. Other reports showed a significant decrease in FEV1 during DBPCFC after doses as low as 120 and 240 mg [97]. Two studies on adults who underwent OFC reported eliciting doses of shrimp of 14 and 16 g [98, 99]. More recently, three patients who underwent OFC with shrimp developed anaphylaxis with doses between 2 and 7.5 g [86]. Doses as low as 11 mg have been reported to elicit symptoms in highly sensitized patients [100].

Dosing protocols for OFC vary. Jirapongsananuruk et al. [86] proposed a three-step protocol with 15-min intervals between doses. Initially, capsules of raw lyophilized shrimp containing 500 mg, 1 g, 2 g, 4 g, and 8 g in 15-min intervals are administered (cumulative dose, 15.5 g). To identify reactions limited to the oral mucosa, 2 g of cooked shrimp is wiped on the inner lips and placed in the mouth without chewing and is spit out after 5 min. If no response is recorded, an open OFC with cooked shrimp in doses of 1, 2, 4, 8, 16, and 32 g at 15-min intervals is performed (cumulative dose, 63 g). Nordlee et al. [100] have proposed a DBPCFC with shrimp incorporated into a seasoned ground beef matrix and cooked. Seven doses ranging from 100 μg to 4 g shrimp were randomly interspersed with three placebo doses. If no response was recorded, an open OFC with shrimp starting at a dose of 4 g and increasing to 16 g of shrimp was performed.

Although there is no standardized initial dose for the OFC, it is recommended to start with a dose lower than that expected to elicit symptoms. EAACI recommends an initial dose of 5 mg for shrimp OFC [95]. Challenge protocols can be scheduled on the basis of logarithmic increases or doubling doses at 15- to 30-min intervals, although the protocols must be individualized to reach the daily recommended dose according to the age of the patient.

Treatment

The mainstay of the treatment of shellfish allergy is the strict avoidance of the offending food. However, accidental reactions can occur, thus patients must be taught to recognize the symptoms, severity, and the treatment they should follow.

As cross-reactivity phenomena are common among shellfish, at least in vitro, once an allergic reaction has occurred with one shellfish species, avoidance of all of them is recommended until clinical tolerance is confirmed in the hospital setting.

Many patients may develop symptoms upon inhalation of cooking vapors, thus avoiding indirect contact with shellfish or cooking vapors is also recommended.

A mutant shrimp TM (Pen a 1 VR9-1) has recently been produced. Substitution of 12 critical amino acids in the eight major IgE-binding regions achieved a 10- to 40-fold reduction in the allergenicity (demonstrated by means of basophil mediator release assays), as compared with wild TM, maintaining its α-helical structure [101], which may be a valid approach for future treatments.

Given the cross-reactivity of shellfish with other invertebrates species, such as mites or insects, allergen-specific immunotherapy may play an immune modulator role that could be potentially curative [102]. Nevertheless, conflicting data have been reported, as shrimp and snail allergy have developed after mite-specific immunotherapy [103–105].

A possible therapeutic approach may be to use linear peptides that correspond to T cell epitopes [77]. In these cases, major epitope-specific T cell lines and clones are produced. These molecular and cellular approaches are advanced in the cases of cat dander, ragweed pollen or bee venom, but no published data on T cell epitopes in shellfish allergy are available. These developing strategies may, in the future,

provide alternative therapeutic tools for shellfish-allergic patients.

Prognosis

Shellfish allergy tends to be persistent; however, scarce data on the evolution of shellfish allergy are available. The only available study dates from 1990, in which the authors followed 11 shrimp allergic children for 2 years and no changes in sIgE titers were found [106].

More recently, differences in the pattern of IgE epitope recognition between children and adults have been reported [107]. A study with 34 children and 19 adult shrimp-allergic patients was carried out. The sIgE levels were shown to be higher in children (47 vs. 12.5 kU/L), and children also recognized a greater number of epitopes and recognized them more intensely than adult patients did. The percentage of children who recognized TM (Lit v 1) was 94 vs. 61 % in adults; MLC (Lit v 3) 70 vs. 31 %, AK (Lit v 2) 67 vs. 21 %, and SCP (Lit v 4) 59 vs. 21 %. The authors suggest that reactivity to shellfish may be lost with age, and sensitization to TM and to some epitopes present in AK and MLC might be associated with persistent sensitization.

Cross-reactivity

Shellfish Cross-reactivity

Although the general impression is that there is a high degree of cross-reactivity between shellfish, there are few studies that address this issue. In the previously mentioned telephone survey [2], 38 % of the participants reported being allergic to crustaceans and 14 % had presented reactions with both crustaceans and mollusks. In 2004, Wu et al. [85] studied patients who reported reactions upon shellfish ingestion by means of SPT. They found that sensitization to bivalves was interdependent and also between shrimp and lobster and limpet and abalone. Significant associations between shrimp and bivalves, crab and limpet, scallop and limpet, and scallop and abalone were also found. Thirty-five percent of the 70 patients that were included in the study were found to be exclusively sensitized to crustaceans, 25.7 % exclusively sensitized to mollusks and 38.6 % were both sensitized to crustaceans and mollusks. According to their data, a patient sensitized to shrimp has 70 % probability of having a positive SPT to crab and 78 % to lobster. This probability diminishes to 49 and 28 %, respectively, in the case of negative results.

Cross-reactivity among mollusks and between mollusks and crustaceans is not well defined. Lehrer et al. [108] demonstrated reactivity to oyster in patients sensitized to shrimp by means of RAST and SPT. Accordingly, sIgE to gastropods, bivalves, and cephalopods was also demonstrated in patients allergic to shrimp [19, 109] and between crustaceans and squid (*T. pacificus*) [46], where TM Tod p 1 [24] was found to be responsible. Tod p 1 also shows cross-reactivity with other squid species (*Loligo vulgaris*), shrimp, lobster, and crab. Cross-reactivity between shrimp and oysters was also shown in a patient with occupational asthma [67]. Nevertheless, the majority of studies on cross-reactivity are based on immunological findings and not on clinical reactivity.

Molecular studies of these allergens suggest that high amino acid sequence homology results in three-dimensional (3D) structure homology as determined by protein folding, and this potentially leads to cross-reactivity [110]. Comparisons of TMs from different crustacean species at the molecular level reveal a high sequence homology, up to 98 %. Nevertheless, homology between shrimp TM and that of mussels or abalone is only 57 and 61 %, respectively. The clinical implications of this cross-reactivity are not well documented as only few studies include oral challenges.

Cross-reactivity between other shellfish allergens has also been documented. Octopus AK has 54 % homology with that of shrimp [31], thus many allergens besides TM are implicated in cross-reactivity among shellfish.

Cross-reactivity With Other Invertebrates

As crustaceans belong to the phylum *Arthropoda*, some studies have addressed the issue of cross-reactivity of crustaceans and other arthropods such as insects or arachnids. TM from *Dermatophagoides pteronyssinus* (Der p 10) shares 65 % of sequence with other invertebrate TMs [111]. Using sera from patients allergic to shrimp, 7/8 epitopes homologous to Pen a 1 have been identified in Der p 10 and Der f 10. It has been suggested that these epitopes are responsible for the cross-reactivity between mites and shrimp [112].

In vivo cross-reactivity is much less frequent than in vitro. In addition, some cases of clinical cross-reactivity have been described in patients allergic to mites who present symptoms upon ingestion of snails [103, 105, 113] and squid [114] or crustaceans [115]. The primary sensitizer is believed to be the mite via inhalation and thereafter, symptoms upon ingestion of shellfish can develop. Conversely, sensitization to mites in children is secondary to crustacean allergy [112].

A multivariate analysis of sIgE to 89 allergens clustered in 12 groups in 1,011 sera [116] revealed that reactivity to shrimp and mussels could be clustered with cockroach but not with dust mites. This analysis suggests that mite TM (Der p 10) is not a major allergen in mites, probably due to the low content of this allergen in mite muscle.

Cross-reactivity with *Anisakis* TM (Ani s 3) has also been documented [46], and this might be due to the high amino acid

sequence homology (74 %) between crustacean TM and Ani s 3 [117].

Nonetheless, cross-reactivity with arthropods is not only due to TM, as there are other allergens that show high sequence homology. This is the case with AK, which is described in invertebrates such as moth (Pol i 1) [60], mites (Blo t 20, Der f 20, Der p 20, and Gly d 20) [61], silkworm (Bomb m 1) [62], spider (Hol pl 9) [63], fruit fly (Dro m 9) [64], and cockroach (Bla g 9 and Per a 9) [65, 66].

Sixty-six percent sequence homology between MLC from shrimp and cockroach (Bla g 8) has also been documented [65], thus it seems that there may be more than one allergen involved in cross-reactivity phenomena, at least in vitro, between shellfish and other arthropods.

Summary/conclusions

Shellfish allergy is increasing in prevalence. Recent advances in the molecular characterization of allergens have led to a better understanding of the allergenic profile in crustaceans and mollusks. To date, many allergens have been characterized: TM, AK, MLC, SCP, and some others. Nevertheless, their clinical relevance is still to be determined. Clinical symptoms are similar to other food allergies, although the inhalation route plays an important role, as some shellfish allergens are capable of aerosolizing. Diagnosis is made by means of SPT, whether with commercial extracts or the prick by prick technique, which have a good NPV. Specific IgE can be determined against many shellfish extracts and recombinant shrimp TM (rPen a 1). The gold standard is DBPCFC. Many dosing protocols are available, although dosing must be scheduled on an individual basis.

Cross-reactivity among shellfish is common, and thus avoidance of all crustaceans and mollusks after the first reaction is mandatory until clinical tolerance is confirmed. In vitro cross-reactivity with other species of the phylum *Arthropoda*, such as mites, is frequent, as they share common allergens. TM and AK have been involved in these phenomena. Nevertheless, clinical cross-reactivity is still a matter of debate.

Although new strategies are being developed, such as the use of mutant shrimp TM or T cell linear peptides, the mainstay of treatment is avoidance of the offending food and recognition of the allergy symptoms and how to establish appropriate treatment.

Further studies are needed to address clinical cross-reactivity with other species as well as possible new therapeutic strategies.

Conflicts of Interest The authors declare no conflicts of interest.

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