

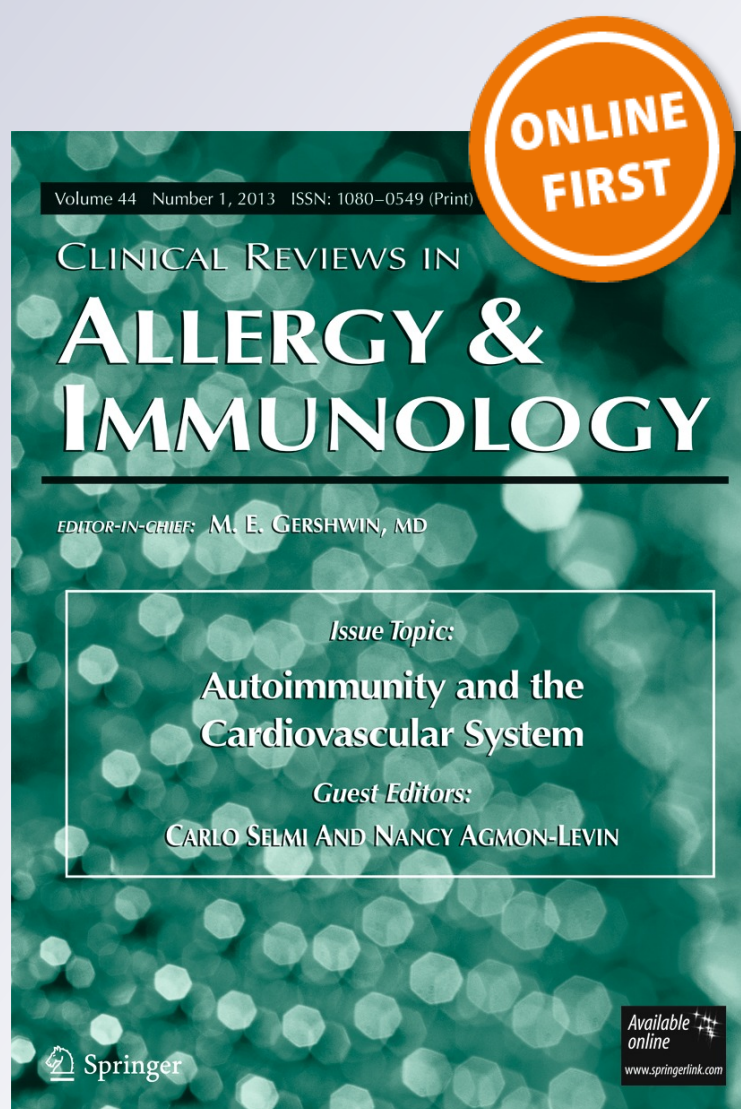
Fish Allergy: In Review

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Fish Allergy: In Review

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Abstract Globally, the rising consumption of fish and its derivatives, due to its nutritional value and divergence of international cuisines, has led to an increase in reports of adverse reactions to fish. Reactions to fish are not only mediated by the immune system causing allergies, but are often caused by various toxins and parasites including ciguatera and *Anisakis*. Allergic reactions to fish can be serious and life threatening and children usually do not outgrow this type of food allergy. The route of exposure is not only restricted to ingestion but include manual handling and inhalation of cooking vapors in the domestic and occupational environment. Prevalence rates of self-reported fish allergy range from 0.2 to 2.29 % in the general population, but can reach up to 8 % among fish processing workers. Fish allergy seems to vary with geographical eating habits, type of fish processing, and fish species exposure. The major fish allergen characterized is parvalbumin in addition to several less well-known allergens. This contemporary review discusses interesting and new findings in the area of fish allergy including demographics, novel allergens identified, immunological mechanisms of sensitization, and innovative approaches in diagnosing and managing this life-long disease.

Keywords Fish allergy · IgE antibody · Food allergen · Prevalence · Clinical symptoms

Introduction

Seafood plays an important role in human nutrition and health, but can provoke serious IgE antibody-mediated adverse reactions in susceptible individuals. A marked increase in

allergic diseases is occurring in most major industrialized countries. The World Allergy Organization reports that in 2008, 20–30 % of the world population was affected by allergy of some type. The seafood allergy and anaphylaxis epidemic is particularly serious. Seafood allergy, including shellfish and fish, is typically life-long affecting up to 5 % of all children and 2 % of all adults. While shellfish and fish allergy are often discussed concurrently, mostly likely due to culinary habits, the allergens causing allergic sensitization are entirely different and must be divided for a comprehensive review. Prevalence rates specifically to fish vary considerably between regions and among children and adults. This review compares the different prevalence rates of fish allergy and explores the possible underlying molecular and immunological causes, resulting in better diagnostic approaches for improved management of this life-long food allergy.

Demographics and Prevalence

Children

Fish allergy has a significant adverse effect on anxiety and stress in the families of affected children. Parental recall of dietary advice is variable and many tend to impose more stringent dietary avoidance than that recommended. Despite this, subsequent accidental reactions are common and demonstrated in over 20 % of diagnosed children [1]. Thus, the avoidance of fish in children may be more difficult than often presumed.

In Europe, most of the populations based prevalence studies come from Spain, Portugal, and the Scandinavian countries. In Norway, adverse food reactions were reported in a population-based study among 3,623 children and nearly 3 % of all reactions were attributed to fish by the age of 2 years [2]. Thus, fish allergy in Norway is almost as common as allergy to egg among children, while fish allergy is more common in children from Finland [3]. A study from

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Spain among 355 children with diagnosed IgE-mediated food allergy reported that fish allergy began predominantly before the second year of life [4].

In the USA, allergy to seafood was reported by about 5.9 % of 14,948 individuals, with about 0.4 % accounting for fish and 0.2 % for both shellfish and fish allergy [5]. The major species reported causing allergic reactions are salmon, tuna, catfish, and cod followed by flounder, halibut, trout, and bass. The majority of allergic subjects reacted to multiple fish species (67 %).

In Australia, a retrospective study in a tertiary clinic among 2,999 children with food allergy demonstrated the prevalence of fish 5.6 %, with white fish, tuna, and salmon being the most implicated fish species [6].

Fish allergy is common, not only in the Western civilization, but also in Asian countries where allergic reactions to fish are significant among children and adults [7] (Table 1). A study from Singapore of 227 children with food hypersensitivity confirmed that fish are significant sensitizers in

approximately 13 % of children. Interestingly, the first intake of fish seems to be very early in life in the Asian diet, with an average age of exposure as low as 7 months. A subsequent prevalence study in the Southeast Asia region used a survey previously developed by Sicherer et al. [5] to compare the occurrence of fish allergy among school children. The population-based study among 11,434 Filipino, 6,498 Singaporean, and 2,034 Thai established that 2.29, 0.26 and 0.29 % of the children suffered from allergic sensitization to fish, respectively [8]. While the prevalence of fish allergy differed between these three Asian countries, females were overall more likely to be sensitized compared to males for all children combined. Nevertheless, most allergies appeared to be of mild nature as less than one third actually sought medical consultation. In most cases, allergic symptoms occurred on first exposure and usually in later childhood. The majority of sensitized Filipino (>50 %) were 11–16 years at the time of first reaction. The most frequently reported fish to cause allergic reactions were anchovy and

Table 1 Epidemiological studies of fish allergy from different continents

Continent	Age	Study subjects	Sample number	Self report (%)	IgE sensitization (%)	Fish studied	References
Asia							
China	<1	Population based	477		0.21	Finfish, conger, whelk, tuna, mackerel, fish roe, anchovy	[8, 17, 108–112]
Hong Kong	2–7	Population based	3,677	0.32			
Philippines	14–16	Population based	11,434	2.29			
Singapore	14–16	Population based	6,498	0.26			
Thailand	14–16	Population based	2,034	0.29			
North America							
Canada	Children	Population based	9,667	0.18		Salmon, catfish, tuna, cod, flounder, halibut, bass, trout	[5, 16]
	Adult			0.56			
	All ages			0.48			
USA	0–17	Population based	3,607	0.17			
	18–67		8,816	0.48			
	All ages		14,948	0.39			
Europe							
Denmark	0.1–22	Birth cohort plus relatives	936		0.52	Fish, codfish	[2, 112–115]
	22–60		898		0.67		
	All ages		1,834		0.60		
France	2–14	Population based	2,716	0.70			
Norway	0–2	Birth cohort	2,803	3.0			
Sweden	0–4	Birth cohort	2,614		0.69		
Turkey	6–9	Population based	2,739	0.33	0.18		
Africa							
South Africa	25–46	Process workers	594		6.00	Hake, yellow tail, salmon, <i>anisakis</i> in fish, canned fish, and fish meal, pilchard, anchovy, snoek	[22, 116, 117]
Australia							
Australia	Children	Allergy clinic patients	2,999	5.6		Barramundi, basa, bream, cod, tuna, salmon, white fish	[1, 6]

mackerel scad. Over one third of sensitized children reported multiple fish allergy, most probably due to the major cross-reactive fish allergen parvalbumin [9–11]. However, the majority of children demonstrated mono-sensitivity to one or the other fish species. Interestingly anchovy and mackerel scad are the first and fifth most common marine fish captured worldwide (Fig. 1), highlighting that other populations with high consumption of these species might be of increased risk of developing fish allergy. There are considerable country-specific differences, which give insights into the impact of cultural behaviors on developing a specific food allergy. While anchovies are used in all three countries to prepare fish sauce, in the Philippines these fish are prepared by drying and salting. This increased immunological reactivity of heated food allergens has previously been described for peanut [12] and also for the fish pilchard [13]. The molecular impact of heating fish allergens is discussed further below. Importantly, children with fish allergy, similar to peanut allergy, will predominately remain clinically reactive throughout their life. A follow-up study by Priftis et al. [14] reported that 65.5 % of fish-sensitized children maintained their sensitization into school age and are at increased risk for wheezing illness and hyperactive airways.

It is to note that prevalence data generated using a survey of self-reported fish allergy are usually higher as when confirmed by specific fish IgE tests. The diagnostic problems and improved approaches are discussed below under “diagnosis and management of fish allergy”.

Adults

A recent study by Vierk et al. [15] provided population-based prevalence data for American adults from a Food Safety Survey of over 4,400 individuals. The prevalence of fish allergy was found to be 0.7 and 0.6 % among respondents with self-reported fish allergy and self-reported

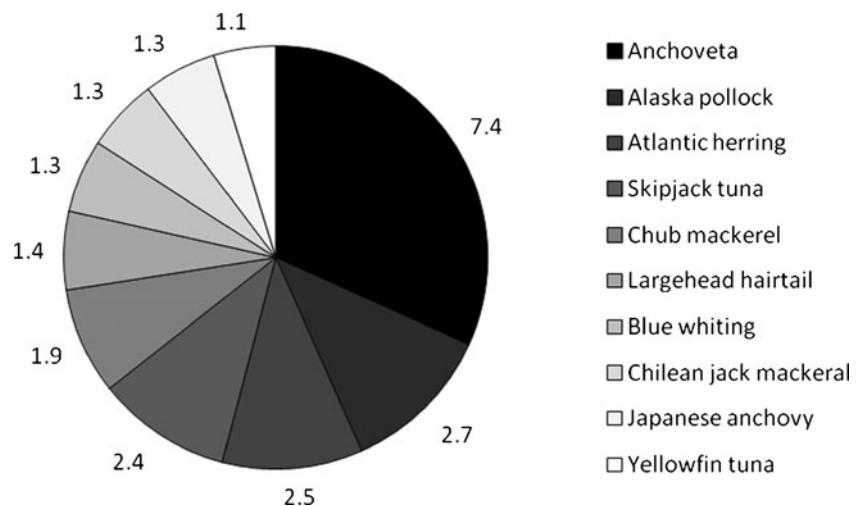
doctor diagnosed fish allergy, respectively. Overall, there was no difference in the prevalence of fish allergy between age or race/ethnic groups. However, significantly more black than white respondents reported a fish allergy. A similar observation was made by Sicherer et al. [5] in a telephone survey of 14,948 individuals with a prevalence of fish allergy of approximately 0.4 %. The reasons for these unexpected observations are not apparent and require further studies among this ethnic group. A recent comparable survey in Canada among 9,667 individuals demonstrated a similar prevalence of fish allergy of 0.51 % [16].

In Asia, fish allergy seems to be also high as documented by a study from Singapore among 74 adults with IgE-mediated food allergy, where fish allergy was with 4.1 % however less common than crustacean allergy (33.8 %) [17].

A study in South Africa determined from a questionnaire of 105 subjects with convincing history of seafood allergy that the four most common bony fish species causing IgE-mediated allergic reactions were hake (24.8 %), yellowtail (21.9 %), salmon (15.2 %) and mackerel (15.2 %) [18]. Clinical symptoms reported included gastrointestinal, respiratory, and dermatological related allergic symptoms. Subsequently, the allergenicity of five fish species was investigated among 10 fish-allergic consumers [9]. Pilchard displayed the strongest IgE reactivity, followed by anchovy, snoek, hake, and yellowtail. Interestingly, most of these IgE reactivities increased after heat treatment [9]. These findings confirmed previous observations on the heat stability and activity of fish allergens [19].

Among adults, exposure to high concentrations of fish allergens and in particular heat-processed fish is particularly observed in various working environments. Occupational sensitization to fish was first reported in 1937 by De Besche in a fisherman who developed allergic symptoms when handling codfish [20]. Since then, various other fish species have been reported to cause occupational allergy and asthma including trout, salmon, pilchard, anchovy, plaice, hake,

Fig. 1 The top ten marine fish captured as of 2008, displayed in million tonnes [25]



tuna, haddock, cod, and pollock [21]. Various studies from South Africa and Norway report the prevalence of occupational asthma between 7 and 8 % [21–24] and protein contact dermatitis from 3 to 11 % [21]. The Food and Agriculture Organization reports that over 45 million people are directly involved in fishery and aquaculture production worldwide (25), making work-related reactions to fish allergens in various contexts an important consideration.

Clinical Features, Exposure Routes, and Mechanisms of Fish Allergy

The main clinical manifestations of allergic reactions to fish include vomiting and diarrhea while the most extreme form of reaction is life-threatening anaphylactic shock (Table 2). Patients with fish allergy can however also react to aerosolized proteins generated by cooking or processing of fish resulting in dyspnea, wheezing, tightness of the throat, urticaria, edema, and light headedness [4, 22, 26–30]. Asthma appears to be a risk factor for fatal anaphylaxis to food [31], and conversely, food allergy is a risk factor for life-threatening asthma [14, 32].

The major route of sensitization to fish is however through the gastrointestinal tract. This mechanism was confirmed for codfish allergens in animal [33] and human studies [34]. The use of antacid medication that increased stomach pH can result in incomplete digestion and thereby increase exposure to and uptake of allergenic fish proteins or peptides. Challenge experiments on patients, without

clinical sensitivity, demonstrated absorption of biologically active fish allergens within 10 min of ingestion. Fish digested at pH3.0 as compared to normal stomach at pH 2.0 revealed comparable reactivity patterns as undigested extracts. However, the nature of the allergen or allergen fragment was not identified in this study. These experiments confirm not only the very high biochemical stability of fish allergens, but also their rapid uptake through the gastrointestinal tract. If patients require antacid medication, this rapid uptake of fish allergens could be of concern and should be discussed with the patient.

In addition to uptake via the gastrointestinal tract, reactions to inhaled proteins are an important aspect of fish allergy in both the domestic and occupational environment. In domestic settings, a Spanish study reported 11 % of children from a group of 197 allergic children experienced repeated allergic reactions upon incidental inhalation of fish odors or vapors, even while on strict fish avoidance. In most cases, these episodes occurred at home when other people were eating fish [28]. Similarly, a South African study of 105 individuals with self-reported seafood allergy, reported 30 % of individuals with allergic symptoms after handling or inhaling seafood in the domestic home environment [35].

In the workplace environment, occupational allergy, and asthma is reported among worker processing a variety of fish species including trout, salmon, pilchard, anchovy, plaice, hake, tuna, haddock, cod, and pollock [21, 36, 37]. Symptoms manifest mainly as upper and lower airway respiratory symptoms and dermatitis, whereas anaphylaxis is rarely seen with this type of exposure. Various studies from

Table 2 Different routes and environments of exposures to fish species and allergens

Route of exposure	Allergen exposure	Domestic	Occupational	Clinical symptoms	Fish species implicated	References
Ingestion	Ingestion of - raw - cooked - processed fish	✓ ✓ ✓		- angiodema - rhinitis - oral allergy - syndrome - urticaria - anaphylaxis - nausea - gastrointestinal	Sea bream, eel, pilchard, salmon, cod	[118–120]
Skin	Dermal contact from - unprotected handling - preparation	✓ ✓	✓ ✓	- urticaria - angiodema	Cod, herring, sardine, swordfish	[121–123]
Inhalation	Inhalation of wet aerosols from - fish heading - degutting - boiling		✓ ✓ ✓	- asthma - rhinitis - skin rash	Plaice, salmon, hake, pilchard, anchovy, tuna, trout, sole, pomfret, yellowfin, salmon	[4, 21, 117, 124]

South Africa and Norway report the prevalence of occupational asthma between 7 and 36 % [21–24] and for occupational protein contact dermatitis from 3 to 11 % [21, 38]. Therefore, work-related reactions to fish allergens in various contexts are an important consideration particularly as it is estimated that up to 15 % of the asthmatic population in the USA and Europe have occupational asthma [39, 40]. Atopy, smoking, and level of exposure are significant risk factors for allergic sensitization and the development of occupational asthma. Fish antigen exposure levels of more than 30 ng/m³ have shown significant correlation with sensitization and work-related asthma symptoms [22]. A similar study quantified raw fish allergens from an open-air fish market and detected allergen concentrations ranging from 2 to 25 ng/m³, very similar to the levels identified in the occupational setting causing allergic sensitization [41]. From data on allergen exposure available so far, it can be expected that extended exposure to aerosolized fish allergens can generate sensitization also in the domestic environment and probably also in children. In general, it is accepted that breaching of oral tolerance leads to food allergy; however, why adults develop de novo food allergy is as yet unknown, and inhalation of fish allergens might be a relevant route of sensitization to consider [42, 43].

A number of fish allergens have been purified and characterized (Table 3) for ingestion-related sensitization. In contrast, the fish proteins in aerosol responsible for allergic sensitization have not yet been fully described [36]. IgE-reactive proteins in fresh, frozen, and canned pilchard range from 12 to 250 kDa. Some of these proteins are identified as monomeric (12 kDa) and oligomeric (36, 48, and 60 kDa) forms of parvalbumin, the major fish allergen in ingestion related allergy. Other fish allergens of importance through the inhalational route might include glyceraldehyde-3-phosphate dehydrogenase, which was recently identified in exposed worker and in a murine model of inhalational fish allergy [13]. In addition to allergens deriving directly from fish tissue, other contaminants such as the fish parasite *Anisakis* have been implicated in occupational sensitization [10, 22, 44, 45]. The major allergen seems to be tropomyosin, which demonstrates cross-reactivity to other invertebrates but not to fish [46, 47]. Future studies need to focus on the molecular characterization of the aerosolized fish allergen causing allergic sensitization and symptoms in the occupational and domestic environment.

It is well recognized that food allergens are in general very heat stable. In addition, it seems that food processing and, in particular, heating can even increase allergenicity as demonstrated for peanuts [48, 49]. Also, the major fish allergen parvalbumin seems to increase its allergenicity as demonstrated in a recent study by Beale et al. [9], where several IgE-binding allergen variants of the major fish allergen parvalbumin were identified in different fish species.

This increased IgE reactivity seems also to be related to stronger allergenicity of this allergen as shown in the subsequent development of the first murine model for inhaled fish allergens [13]. Heat-treated pilchard allergens significantly increased Th2 cytokines and specific IgE responses as compared to untreated allergens. In contrast, raw pilchard allergens initiated a specific IgE response to a novel fish allergen, glyceraldehyde-3-phosphate dehydrogenase. Interestingly sensitized fish processing workers also recognized this IgE reactive allergen. This murine model of inhalational fish allergy demonstrated for the first time that inhalation exposure to fish allergens can generate a strong IgE-mediated allergic sensitization to parvalbumin.

This deep insight into the mechanism of inhaled fish allergy and the enhanced response to heat-treated parvalbumin is supported by recent studies on human cells. Enhanced internalization of glycated allergens, such as ovalbumin, was recently studied in human dendritic cells, which led to increased CD4⁺ T-cell immunogenicity of this protein [50, 51]. Heating of proteins in the presence of sugars such as glucose, result in so-called “advanced glycation endproducts” (AGEs), through the Maillard reaction. These AGEs seem to stimulate the uptake of allergens by antigen-presenting cells through binding to scavenger receptors. In summary, these studies give strong indications that heated fish allergens are more allergenic than their unheated counterparts and this could be of considerable importance for better diagnostics but also the development of novel therapeutics for this type of food allergy.

Classification of Fish

Fish species can be divided into two main groups; the bony fish and cartilaginous fish. Most edible fish belong to the bony fish (Osteichthyes), whereas sharks and rays are cartilaginous and belong to a different class; Chondrichthyes. Most studies on fish allergens have focused on cod, carp, and salmon [52–58]. Although there are more than 32,400 different species of fish described [59], consumption depends heavily on regional availability and can include under investigated fish such as basa, barramundi, and elephant shark.

The class of bony fish can be further divided into 45 orders. The most commonly consumed bony fish belong to the orders Clupeiformes (herrings and sardines), Salmoniformes (salmons and trouts), Cypriniformes (carps), Gadiformes (cods, hakes, and whiting), Siluriformes (catfish), and Perciformes (perches, mackerels, and tunas). The later order Perciformes itself comprises 156 diverse families and is the largest order of vertebrates with over 9,300 species [59]. The top marine fish species captured include representatives from most of these orders (Fig. 1)

Table 3 Selection of allergenic proteins characterized in 24 fish species representing 9 different orders and their biochemical characteristics (PV parvalbumin)

Common name	Scientific name	Order	Allergen identified isoform	MW (kDa)	References
Atlantic mackerel	<i>Scomber scombrus</i>	Perciformes	PV β	11.5	[125]
Big eye tuna	<i>Thunnus obesus</i>	Perciformes	Collagen	120–240	[77]
Chub mackerel	<i>Scomber japonicus</i>	Perciformes	PV β	11.5	[125]
Japanese jack mackerel	<i>Trachurus japonicus</i>	Perciformes	PV β	11.3	[126]
Skipjack tuna	<i>Katsuwonus pelamis</i>	Perciformes	PV β	11.4	[70]
Swordfish	<i>Xiphias gladius</i>	Perciformes	PV β	11.5	[127]
Yellowfin tuna	<i>Thunnus albacares</i>	Perciformes	PV β	11.5	[11]
			Enolase β	47.1	
			Aldolase		
Alaska pollock	<i>Theragra charlcogamma</i>	Gadiformes	PV β	11.5	[55]
Atlantic cod	<i>Gadus morhua</i>	Gadiformes	PV β 1	11.5	[52]
			PV β 2	11.5	[52]
			PV β 3	11.5	[128]
			PV β 4	11.5	[128]
			Enolase β		
			Aldolase		
Baltic cod	<i>Gadus callarias</i>	Gadiformes	PV β	12.1	[54]
European hake	<i>Merluccius merluccius</i>	Gadiformes	PV β	11.3	[9, 129]
Atlantic herring	<i>Clupea harengus</i>	Clupeiformes	PV β 1	11.7	[91]
			PV β 2	11.7	[91]
			PV β 3	11.8	[91]
Japanese sardine	<i>Sardinops melanostictus</i>	Clupeiformes	PV β	11.9	[19, 70]
Pacific pilchard	<i>Sardinops sagax</i>	Clupeiformes	PV β	11.9	[9]
Atlantic salmon	<i>Salmo salar</i>	Salmoniformes	PV β 1	11.9	[58]
			Enolase β	47.3	
			Aldolase		
Rainbow trout	<i>Oncorhynchus mykiss</i>	Salmoniformes	PV β 1	11.8	[130]
			PV β 1	11.3	
			Vitellogenin	–	
Japanese flounder	<i>Paralichthys olivaceus</i>	Pleuronectiformes	PV β	11.6	[70, 126]
Whiff	<i>Lepidorhombus whiffiagonis</i>	Pleuronectiformes	PV β	11.7	[131]
Beluga	<i>Huso huso</i>	Acipenseriformes	Vitellogenin	118	[76]
Carp	<i>Cyprinus carpio</i>	Cypriniformes	PV β	11.5	[53, 63, 132]
Japanese eel	<i>Anguilla japonicus</i>	Anguilliformes	PV β	11.7	[70]
Rose fish	<i>Sebastes marinus</i>	Scorpaeniformes	PV β 1	11.4	[133]
			PV β 2	11.7	

[25]. However, less than 0.5 % of all known fish species has been analyzed for their allergens on molecular level and demonstrates unexpected large diversities as detailed below.

Fish Allergens

The Major Fish Allergen Parvalbumin

The Baltic cod was the first food source in the early 1970s ever analyzed for the molecular nature of the offending

allergen. The major allergen identified was subsequently named Gad c 1, a parvalbumin protein that regulates calcium switching in muscular skeletal cells [60–62]. Parvalbumin represents the major clinical cross-reactive fish allergen with 90 % of fish allergic patients reacting to this protein [53, 63, 64]. Furthermore, this allergen forms the biggest group of animal derived food allergens, the EF hand domain family (<http://www.meduniwien.ac.at/allergens/allfam/>), with over 63 allergens currently reported.

Parvalbumin is not only present in lower vertebrates such as fish and frog, where it can be an allergen [65, 66], but is

also found in higher vertebrates including humans, demonstrating that parvalbumin plays a vital role in basic vertebrate calcium physiology [67]. Parvalbumins can be found as one of two distinct isoform lineages; α and β . Fish often contain both α and β parvalbumin; however, the majority of allergenic parvalbumin's reported belong to the β lineage (Table 3). Furthermore, most fish express two or more different β parvalbumin isoforms, which are subsequently named $\beta 1$, $\beta 2$, and so forth [52]. These β isoforms can differ significantly in amino acid sequence as demonstrated for Atlantic salmon (*Salmo salar*) where their $\beta 1$ and $\beta 2$ isoforms have only 64 % identity. The differences in β parvalbumin isoforms in one species can result in a fish allergic patient reacting to one isoform more than another, which adds to the complexity of diagnosing fish allergy and detecting allergenic parvalbumin [57]. In addition, dimeric as well as polymeric forms of parvalbumin have also been reported to bind IgE antibody and these allergens form higher molecular weight aggregates of approximately 24 and 48 kDa [68, 69]. The allergenicity of parvalbumin has been studied in a number of fish species and as of 2012, the allergome database (www.allergome.org) has 218 allergenic isoforms of fish parvalbumin listed, while only 27 of these isoforms are actually registered with the World Health Organization (WHO) or International Union of Immunological Societies (IUIS). This registration substantiates the prevalence and specific molecular nature of this allergen according to specific guidelines by WHO and IUIS and has only been achieved for just over 10 % of all current studies. More detailed molecular studies on fish allergens will assist in the development of better diagnostics and potential immunotherapeutics.

IgE Epitopes and Cross-Reactivity

Thus far, there have been four attempts to identify the IgE epitopes of allergenic parvalbumins (Fig. 2a). Parvalbumin from Baltic cod (*Gad c 1*), carp (*Cyp c 1*), chub mackerel (*Sco j 1*), and Atlantic salmon (*Sal s 1*) were analyzed for their specific IgE epitopes. Allergic patient IgE was used in various techniques including phage display library, overlapping immunogenic peptides, and tryptic digests of parvalbumin to map out these epitopes [54, 56, 57, 70]. These four fish parvalbumin display both linear and conformational epitopes, however do not share identical residues. This may be due to the polyclonal nature of IgE antibodies from different patients as well as the varying techniques utilized to identify these epitopes. In summary, the four parvalbumin allergens currently analyzed on molecular level demonstrate very different IgE binding epitopes [10, 56, 57, 63]. While the secondary and tertiary structures of parvalbumins are highly conserved among fish, their primary structure, or amino acid sequence, differs substantially.

Epitope alignment of these four fish parvalbumins, using two different computer models, allows the identification of highly antigenic (region IV) in contrast to species-specific proteins regions (region I). Indeed, the later can be confirmed by reports of monosensitivity to salmonids [71, 72]. This phenomenon could account for fish allergy sufferers having only about a 50 % chance of being cross-reactive to another fish species [73] and is significantly lower than the rate of shellfish cross-reactivity which is up to 75 % [73, 74]. Further studies need to confirm that the identified protein region IV is responsible for sensitivity to multiple fish species and would be of great importance for improved diagnostics.

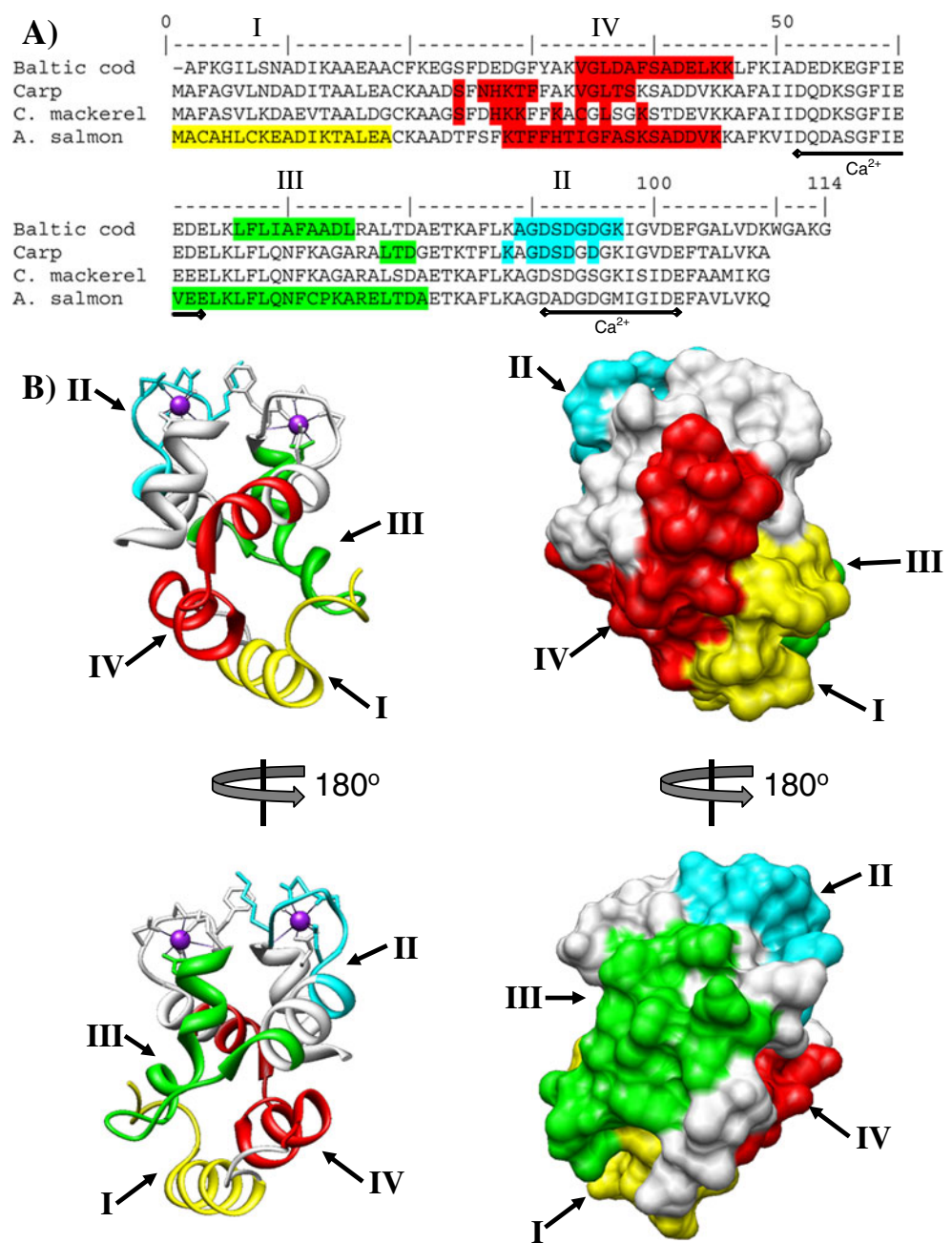
Other Fish Allergens

In addition to parvalbumin, other fish allergens have been characterized such as the hormone vitellogenin from Beluga caviar [75, 76] and collagen and gelatin isolated from skin [77, 78] and muscle tissues of fish [79]. The allergenicity of isinglass derived from fish swim bladder used for filtering beer has also been investigated, demonstrating that the gelatin content of isinglass to be harmless to fish allergic subjects. However, small amount of allergenic parvalbumins were detected in isinglass at levels up to 414.7 mg/kg which might be of importance for very sensitive patients [80]. In addition, enzymes such β -enolase and aldolase from cod, Atlantic salmon, and tuna have been submitted to WHO and IUIS as fish allergens. It is to note that there seems to be no cross-reactive allergens between fish and shellfish [10, 74]. In addition to these allergens derived from fish themselves, contaminants such as the parasite *Anisakis* can cause allergic reactions [10, 81]. Exposure to proteins from live or dead *Anisakis* can cause allergic reactions. The 13 allergens characterized in *Anisakis* include tropomyosin, as well as paramyosin and protease inhibitors. Allergens from *Anisakis* appear not to be destroyed by heat or cooking and so allergic reactions may be triggered by dead parasites in fish that have been well cooked. A recent study demonstrated that these parasites can also cause considerable allergic sensitization among fish processing workers [45, 82]. While the identified allergens seen not to cross-react to fish allergens, possible allergic reactions to ingested fish could be directed to the contaminating parasite *Anisakis* and be falsely diagnosed as fish allergy [10].

Non-IgE-Mediated Reactions to Fish

Adverse reactions to fish can also be mediated by non-immunological reactions in contrast to true food allergy [83, 84]. These reactions can result from exposure to fish itself or various non-fish components in the product. Non-

Fig. 2 a Amino acid sequence alignment of Baltic cod (Gad c 1.01 UniProtKB Accession number: P02622), carp (Cyp c 1.01 UniProtKB Accession number: E0WD92), chub mackerel (Sco j 1.01 UniProtKB Accession number: P59747) and Atlantic salmon (Sal S 1.01 UniProtKB Accession number: B5DH15). Known IgE epitopes are located in four regions colored *yellow*, *blue*, *green*, and *red*. These regions are labeled I, II, III, and IV according to the number of fish species sharing the same IgE binding region. The two calcium-binding sites of this muscle protein are *underlined*. **b** Ribbon and space filling carp parvalbumin models (Protein Data Base ID: 4cpv) with the four epitope regions colored and labeled according to section a. *Purple* bound calcium



immunological reactions to fish can be triggered by contaminants such as bacteria, viruses, marine toxins, parasites, and biogenic amines. The latter is mostly found in “spoiled” fish (scombroid poisoning) [85, 86]. Marine biotoxins, generated by algae, can be detected in fish [10, 74, 83] and also in filter feeders such as mussels and oysters. Eating fish that has been contaminated by algae-derived toxins in particular causes Ciguatera poisoning. Ciguatera toxins are only present in fish, particularly large reef fish in the tropics. These toxins interfere with the function of nerve endings with symptoms occurring within 2–3 h of eating contaminated fish, and consist of tingling of the lips, tongue, and throat and sometimes change in blood pressure and heart rhythm.

Most people recover within a few days or weeks with supportive treatment.

Contamination of fish with parasites can also cause severe adverse reactions as in the case of *Anisakis* simplex, a parasitic nematode that is found in most parts of the world [87, 88]. *Anisakis* can cause two major problems in humans: Infections with live *Anisakis* (anisakiasis) can result from eating raw, pickled, or undercooked fish. Infection may cause nausea, vomiting, stomach pain, and sometimes appendicitis, bowel blockage, or bleeding.

Finally ingredients, such as spices and monosodium glutamate, added during processing and canning of fish can also cause adverse reactions. Importantly, all of these

substances can trigger clinical symptoms, which are similar to true allergic reactions including respiratory symptoms, urticaria, and headache. Due to this similarity in clinical reactions of affected consumer and worker, it is of critical importance to differentiate adverse reactions from true fish allergy and comprehend the underlying mechanisms of allergic reactions and molecular nature of these allergens. Adverse reactions to fish are however too manifold to be discussed in detail in this review and referred to other articles [10, 89, 90].

Diagnosis and Management of Fish Allergy

In vitro diagnostic methods of fish allergy include in vivo skin prick test (SPT) as well as in vitro quantification of specific IgE antibodies using assays such as the ImmunoCAP (Thermo Fisher) and immunoblotting to identify the specific IgE binding allergens. One example of commercial in vitro assays to quantify specific IgE to allergens is the ImmunoCAP system, which offers currently 27 different fish species and two recombinant fish allergens from carp and cod. However, a direct comparison of all these fish species for their IgE reactivity has not been conducted. While these types of assays contain the majority of possible allergens found in the individual fish species, possible variations of parvalbumin concentrations cannot be taken into account. These parvalbumin variations have recently been analyzed in seven fish species by Kuehn et al. [91] and demonstrated over tenfold lower concentrations of the major fish allergen in tuna compared to herring, which could impact on the sensitivity of various diagnostic tests.

It is well accepted that the level of serum IgE antibodies is directly related to the severity of allergic reactions and previous studies by Sampson et al. [92] tried to predict clinical reactivity based on specific IgE levels. For cod–fish, a diagnostic level of IgE that can predict clinical reactivity in a US population, with >95 % certainty, was identified as 20 KUA/l. It is however questionable if this seemingly high value can be extrapolated to other fish species and other populations as IgE values as low as 1 kU/l could be determined in patients with anaphylactic reactions to pilchard and anchovy [9].

Patients who generate IgE antibodies to one parvalbumin often react to parvalbumin of other fish species, demonstrating the importance of parvalbumin as a cross-reactive major fish allergen [63]. Approximately one third of children and two thirds of adults appear to react to multiple types of fish [5, 64, 93, 94]. Van Do et al. [11] demonstrated in 10 patients, using a combination of SPT, ImmunoCAP, and immunoblotting, that Gad c 1, Sal s 1, The c 1, herring, and wolffish contained the most potent cross-reacting allergens, whereas halibut, flounder, tuna, and mackerel were the

least allergenic in the current study. It is suggested that the latter fish species could probably be tolerated by some of the tested patients. However, allergic reactions to only one specific type of fish have been reported such as in salmonids where patients react to trout and salmon but not to cod, carp, herring, or redfish [71, 72]. Asero et al. [95] described a patient that was monosensitive to tropical sole but did not react to lemon sole, cod, salmon, tuna, and swordfish. Fish monosensitivity has also been seen in a patient who reacted to tilapia and pangasius, but not to cod. Subsequent analysis showed that the patient reacted to an unknown allergen but not to parvalbumin [96]. These few studies demonstrate that monosensitivity to fish is not uncommon and most prominent in children; however, the molecular nature of responsible allergens is yet to be fully elucidated. Recent findings by Gill et al. [97] indicate that reactivity to specific allergens is associated with disease risk, confirming the importance of molecular identification of causative allergens.

SPT is frequently used as a first test to confirm or refute allergic reactions to fish as it provides a rapid, safe, and inexpensive method for screening patients. Nevertheless, these types of tests are considered to be not very specific with a positive predictive value often below 50 % [98]. Skin prick tests are of particular challenge for fish allergy due to the large variety of fish species being implicated and the fact that the majority of patients seem to demonstrate monosensitivity to specific species. To improve the specificity and sensitivity of this test, Van do [11] compared the SPT reactivity of the recombinant with the natural parvalbumins from salmon, cod, and pollock. Surprisingly, only one of the ten patients recognized the recombinant versions of the natural parvalbumins, which were in contrast recognized by nine of the patients. The poor response obtained in using recombinant parvalbumin in SPT is possible due to conformational masking of high-affinity IgE-binding motifs (Fig. 2b). These studies suggest that the IgE reactivity to recombinant parvalbumin has to be investigated in more detail in future studies to use these allergens in in vitro and in vivo tests.

The gold standard for diagnosing food allergy is still the double-blind, placebo-controlled food challenge. A recent review by Niggemann and Beyer [99] proposes various decision trees to approach food challenges including seafood-allergic patients. A similar decision tree is suggested for fish allergy, which includes non-immunological adverse reactions triggered by toxins and parasites (Fig. 3). There is limited information on the establishment of threshold values for elucidating allergic reactions to fish. A recent study [34] demonstrated that for codfish, very small amounts of less than 3 mg protein could trigger allergic reactions, which is less than previously reported [100]. A similar quantity was used to confirm allergy to yellowtail in a study on seven fish allergic patients from South Africa [18].

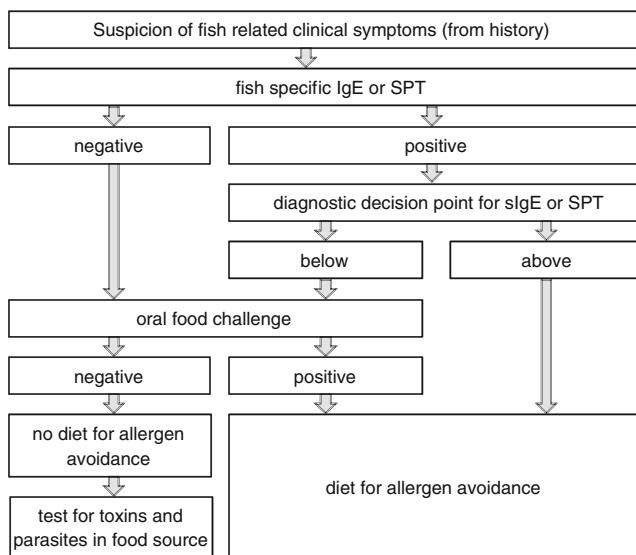


Fig. 3 Diagnostic decision tree on how to proceed from the suspicion of fish-related allergic symptoms, modified from Niggemann et al. [99] and Mehl et al. [134]

One target for the development of immunotherapeutics for fish allergy is the muscle protein parvalbumin, which is the major allergen recognized by over 90 % of patients with fish allergies [10, 63, 101–103]. The major IgE-binding epitopes of parvalbumin are considered to be conformational epitopes as detailed in the studies listed above [56]. These antibody epitopes appear to be dependent on the functional reactivity of the binding sites for Ca^{2+} and Mg^{2+} . Conformational changes in these protein regions using recombinant technologies can result in hypoallergenic parvalbumin as has been recently demonstrated for carp [102]. Although still immunogenic, as demonstrated through specific IgG responses in mice, the reactivity measured by SPT in patients was markedly reduced. This novel hypoallergenic protein forms the basis for safer novel forms of future vaccination against fish allergy. Nevertheless, it has to be highlighted that the immunological reactivity of recombinant allergens are not necessarily identical to native allergens. Van der Ventel et al. [13] demonstrated in an inhalant murine model that the recombinant parvalbumin from carp is not as reactive as parvalbumin from pilchard. In addition, heated parvalbumin was much more allergenic than raw parvalbumin and other allergens, in addition to parvalbumin, seem to be relevant.

While heating appears to increase allergenicity of some of the fish allergens, commercial heat processes, used to generate canned fish, seem to have a different effect. A recent descriptive study from Australia demonstrated that more than 20 % of children allergic to salmon or tuna were able to tolerate the fish in canned form. Importantly this was associated with a reduction in SPT size in most patients, implying that the consumption of canned fish may have resulted in the induction of tolerance in these patients [104].

While immunotherapy for fish allergy is still in development, management of fish allergy is generally directed at avoidance of the offending foods and prompt recognition and treatment of acute allergic reactions. In addition, reactions to hidden food allergens through inhalation of the fish allergens or via skin contact can also pose problems [26, 28]. In a recent study, 22.7 % of 530 food-related reactions were due to hidden allergens with 35 % of fish allergic patients having reacted to fish proteins hidden in other foods or to fish vapors [26].

In general, management of food allergies, including fish allergy, still primarily relies on avoidance. The labeling of foods containing materials derived from fish has already become mandatory in some countries such as the USA, Europe, and Japan. While in vitro assays for currently 14 food allergens in the EU are available, the detection of parvalbumin is much more problematic as these allergens show very high biochemical and immunological variability among the different fish species as detailed above [105, 106]. Currently, there is only one commercial test available to detect the presence of fish DNA, but is limited to 12 fish species (www.r-biopharm.com). Labeling regulations have limitations because of accidental cross-contamination with allergens through shared equipment in production lines or the unknown presence of a hidden fish allergen such as clarification agents derived from fish bladders used in wine and beer [107].

Conclusion

Fish allergy has a significant adverse effect on anxiety and stress among adults but also in families with allergic children. There seems to be strong geographical differences in the prevalence of fish allergy, possible due to different cultural dietary habits and type of food processing. The later might even enhance allergenicity of fish allergens due to advanced glycation end products as demonstrated in vitro and utilizing murine models. More detailed immunological studies are needed to characterize the impact of heating on fish allergens to develop better food processing technologies to reduce their allergenicity.

The majority of allergic reactions to fish are caused by the major allergen parvalbumin. Immunological cross-reactivity between the vast variety of fish species seems to be determined by the degree of amino acid homology and in addition number of allergen isoforms and variants present in some of the highly allergenic species. In addition, the concentration of this major allergen varies significantly among the different fish species and might impact on patients' sensitivity to one or multiple species. Future comparative studies need to investigate the molecular and immunological similarity of parvalbumins among the different fish groups

and families, with focus on B- and T-cell epitopes, to allow the generation of group-specific recombinant allergens for better identification of patients with multiple fish reactivity.

The route of sensitization to fish allergens seems to initiate differential immunological reactions to additional allergens as demonstrated in the occupational environment, which needs to be addressed in the diagnosis of fish allergy.

The current diagnosis and management of fish allergy are hampered by the lack of detailed information of the molecular nature of these allergens, the enormous variety of allergenic fish species consumed and the subsequent lack of suitable tests to detect specific allergens in food products. In the absence of suitable commercial SPT or IgE assays to a specific fish species it is suggested to quantify specific IgE to Atlantic cod, Atlantic salmon, Pacific pilchard, and European hake, as these four species cover the broad molecular spectrum of the major allergen parvalbumin. In addition lipopolysaccharide free protein extracts, preferably raw and heat-treated, of the specific fish species could be used for SPT.

Future comparative studies on the clinical reactivity to various fish species among different populations will improve diagnosis and management of this life-long allergy. The development of better recombinant and hypoallergenic parvalbumin's is an important basis for more sensitive and specific in vivo and in vitro diagnostics and safer novel forms of vaccination against fish allergy.

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