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Food Allergy (M Fernandez-Rivas, Section Editor)

Fish Allergy Management: From Component-Resolved Diagnosis to Unmet Diagnostic Needs

Julia Klueber, MSc[1,2]
Denise Schrama, MSc[3]
Pedro Rodrigues, PhD[3]
Heinrich Dickel, MD[4]
Annette Kuehn, PhD[1,*]

Address
[*,1]Department of Infection and Immunity, Luxembourg Institute of Health, 29, rue Henri Koch, L-4354, Esch-sur-Alzette, Luxembourg
Email: annette.kuehn@lih.lu
[2]Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, University of Southern Denmark, Odense C, Denmark
[3]CCMAR, Centre of Marine Sciences, University of Algarve, Faro, Portugal
[4]Department of Dermatology, Venereology and Allergology, St. Josef-Hospital, University Hospital of the Ruhr -University Bochum (UK RUB), Bochum, Germany

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Abstract

Purpose of review Fish is a common elicitor of IgE-mediated food allergy. Fish includes a large variety of foods, in terms of species and food processing, with marked distinction in local diets around the globe. Fish-allergic patients present with phenotypic diversity and major differences in levels of clinical cross-reactivity, features that pose an important challenge for the clinical diagnosis and management.
Recent findings Parvalbumin is the major fish allergen. However, a single molecule is not sufficient but several homologs, allergens different from parvalbumin and allergen extracts, are needed for IgE-based diagnosis.
Summary Parvalbumin-specific IgE are markers for clinical cross-reactions. Added value is provided by IgE typing to parvalbumin homologs from distantly related fish. IgE co-sensitization profiles (parvalbumin, enolase, aldolase) are referred as severity markers. The allergen panel seems to be not yet complete why fish extracts still play a crucial role in

serum IgE analysis. Further clinical validation of a multiplex approach in molecular fish allergy diagnosis is needed for striving to avoid unnecessary food restrictions and in a further sense, improved patient care.

Introduction

Fish is a diverse food that is popular in many human diets around the globe and beyond, it is considered to be a healthy alternative to meat. The ingestion of fish or contact with fish can be a source for adverse reactions while IgE-mediated allergies are considered the most common type. In addition to milk, eggs, peanuts, tree nuts, soy, wheat and seafood, fish is counted among the most frequent triggers of IgE-mediated food allergies [1]. Fish is also an important cause of occupational allergies [2, 3]. Clinical symptoms involve single or several organs, ranging from mild to severe anaphylaxis. As an animal food source, fish is highly diverse exhibiting the largest species diversity among vertebrates. Allergenic molecules vary in different fishes. This, along with various fish preparation methods causing allergen modifications, causes human exposure to a broad variety of intact and modified allergens. Fish-allergic patients are characterized by phenotypic diversity, with major differences in levels of clinical cross-reactivity (e.g. fish-fish). This article will provide an overview about the current state-of-knowledge around IgE-mediated fish allergy and the most important fish allergens. Diagnostic challenges related to the molecular component diagnostic approach will be discussed in the light of unmet medical needs.

Fish: healthy variety on the plate

Fish is an important supplier of protective omega-3 fatty acids, high levels of protein, various trace elements and lipid-soluble vitamins. A total of over 700 fish species are available commercially, most of the species are bony fishes (*Osteichthyes*) [4]. Frequently consumed edible fish belong to the following families: salmons (e.g. Atlantic/Pacific salmon, trout), cod-like fish (e.g. Atlantic cod, Alaska pollock), flatfishes (e.g. plaice, sole), perch-like fish (e.g. tuna, mackerel, swordfish), herring-like fish (e.g. herring, sardine, anchovy), carp-like fish (e.g. carp, barbel) and catfish-like (e.g. catfish, pangasius) [5••]. Bony fish have mostly light muscle tissue; typical dark muscle fishes are pelagic species like tuna, herring and mackerel (www.fao.org). The light muscle is adapted to rapid movements, the dark muscle to continuous long-range swimming. Cartilaginous fish (*Chondrichthyes*), rays and sharks are distant relatives of bony fish [6•]. While bony fish satisfy the global market, the consumption of cartilaginous fish is limited to specific regions. According to estimates by the Food and Agriculture Organization of the United Nations (FAO), there is a growing demand worldwide for fish and fishery products. The consumption of fish varies greatly over the world, depending on eating habits and local supply (e.g. ca. 22 kg/capita/year in Europe or Northern America; ca. 42 kg/capita/year in China). Consumed fish species vary in different geographical regions. Cod, salmon, tuna and Alaska pollock are among the top species in Europe while in Asia-Pacific regions, others are popular (e.g. tilapia, catfish, perch and snakehead) (www.fao.org). Furthermore, preparation methods vary widely, from raw over to strongly processed fish. Variable global patterns of fish

consumption (e.g. species, processing) entail a wide spectrum of fish antigens in human exposure.

Beyond IgE-mediated fish allergy

Different types of adverse reactions to fish are known, immunological and non-immunological reactions. Briefly, the following categories need to be distinguished from the classical type of genuine type I hypersensitivity.

Food protein-induced enterocolitis syndrome

This cellular type of food allergy manifests with delayed (1–4 h) gastrointestinal symptoms after ingestion [7]. Among others (e.g. cow's milk, soy, rice), fish belongs to the most prevalent foods triggering food protein-induced enterocolitis syndrome (FPIES), especially in the Mediterranean area [8, 9]. The clinical diagnosis is based on a detailed medical history (e.g. clinical symptoms, timing). No laboratory tests are available to confirm fish-related FPIES diagnosis.

Fish allergy-like symptoms by toxins

Specific fish, such as tuna or mackerel (scombroids), contain high muscle levels of histidine. Upon bacterial fish spoiling, histidine decomposition results in the histamine formation [10]. Histamine might be also formed during fish processing such as canning. The intake of the histamine-rich fish induces allergy-like symptoms [11]. The diagnostic work-up relies on the clinical history including absence of specific IgE and low reproducibility of the adverse reaction.

Anisakis-induced symptoms

Anisakis is a parasitic worm infecting fish muscle. Anisakis-spoiled fish, such as in sushi, can induce human illness (anisakiasis) with inflammatory intestinal symptoms [12, 13]. Anisakis is also causing IgE-mediated allergy to helminth allergens, mostly excretion/secretion parasite molecules [14]. It has been reported that only the ingestion of life parasites leads to clinical symptoms [15]. The medical diagnosis of Anisakis-induced adverse reaction is based on the anamnesis (clinical manifestation, fish preparation, reaction reproducibility), as well as in the case of type I food allergy, serum IgE testing with Anisakis extract.

IgE-mediated fish allergy in review

Epidemiology

Fish is one of the most common triggers of IgE-mediated food allergies. Questionnaire-based studies revealed prevalence rates of 0 to 7% (e.g. USA 0.2%, Greece 1.5%, Finland 7%). Allergic sensitization (skin, serum)

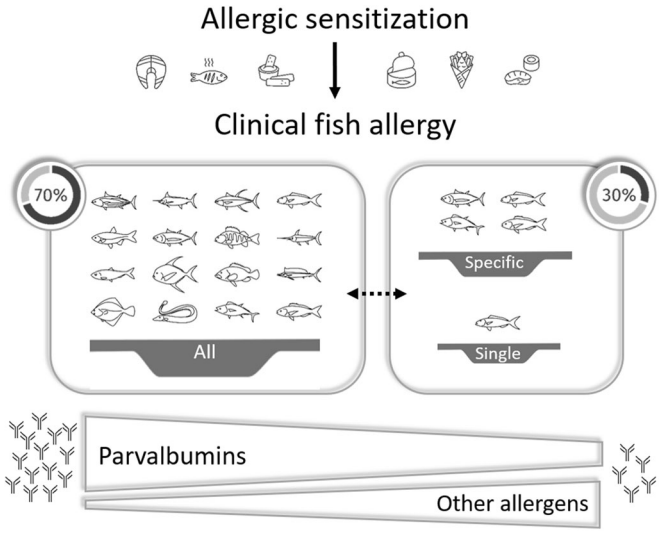

Fig. 1. Allergic sensitization to fish is primed by dietary conditions (species, processing). Patients feature broad to limited cross-reactivity in 70% and 30%, respectively, of the cases. Parvalbumins are important cross-reactivity markers, with higher IgE-titers in patients of broad as compared with those of low cross-reactivity.

identified up to 2.9% of the individuals (e.g. China 0.2%, Norway 1.1%, Germany 2.9%). Finally, food challenge-confirmed prevalence rates range up to 0.3% (e.g. Denmark 0.2%, Iceland 0.3%) [16]. The prevalence of fish allergy is higher in regions with frequent fish exposure (diet, processing industries). Of note, prevalence datasets provide an informative basis but are specific for the underlying study conditions (e.g. participants' age, fish species in food challenge) [17, 18].

Pathogenesis

Early events play a critical role for immune development, both tolerance development and breakdown [19]. Whether early introduction (before 12 months of age) does contribute to prevent fish allergy still needs further investigation [20]. Including fish into the diet of young infants seems to reduce the risk for developing asthma and allergic rhinitis [21, 22]. Many patients develop clinical fish allergy during childhood that persist during adulthood [16]. Adulthood onset of fish allergy, both as classical food allergy and occupational allergy, is also known. Clinical symptoms occur within minutes after fish ingestion, inhalation and skin contact, respectively, leading to cutaneous (urticaria, angioedema), gastrointestinal (oral allergy syndrome, laryngeal edema, spasm, diarrhea, vomiting) and respiratory manifestation (rhino conjunctivitis, bronchospasm) as well as in some cases, severe anaphylaxis [3, 23••, 24].

Clinical reactivity profiles

Patients react primarily to fish that are part of their diet. Still, those individuals have a considerable risk of developing adverse symptoms with several, if not all, species of fish. Clinical cross-reactivity was considered

Table 1. Bony fish muscle extracts and molecules available in commercial sera IgE assays, approved WHO/IUIS allergens and proposed current CRD-panel as a systematic approach

			Commercial IgE-test*		WHO/IUIS allergens	CRD-approach**
Anguilliformes	Eel	*Anguilla anguilla*	Extract	-	-	
Clupeiformes	Herring	*Clupea harengus*	Extract	-	Clu h 1	Clu h 1 or Sar sa 1
	Sardine	*Sardina pilchardus*	Extract	-	-	
	Sardine	*Sardina sagax*	-	-	Sar sa 1	
	Japanese pilchard	*Sardinops melanosticta*	Extract	-	-	
	Anchovy	*Engraulis encrasicolus*	Extract	-	-	
Salmoniformes	Salmon	*Salmo salar*	Extract	-	Sal s 1	Sal s 1
					Sal s 2	
					Sal s 3	
					Sal s 6	
	Trout	*Oncorhynchus mykiss*	Extract	-	Onc m 1	
	Whitefish	*Stenodus sp*	Extract	-		
Gadiformes	Atlantic cod	*Gadus morhua*	Extract	rGad c 1	Gad m 1	Gad m 1
					Gad m 2	Gad m 2
					Gad m 3	Gad m 3
	Baltic cod	*Gadus morhua*	-	-	Gad c 1	
	Pollock	*Pollachius virens*	Extract	-	-	
	Hake	*Merluccius merluccius*	Extract	-	-	
	Haddock	*Melanogrammus aeglefinus*	Extract	-	-	
Lopiiformes	Angler	*Lophius piscatorius*	-	-	-	
Cypriniformes	Carp	*Cyprinus carpio*	-	rCyp c 1	Cyp c 1	
	Grass carp	*Ctenopharyngodon idella*	-	-	Cten i 1	
Siluriformes	Catfish	*Ictalurus punctatus*	Extract	-	-	
	Pangasius	*Pangasianodon hypophthalmus*	-	-	Pan h 1	
Carangiformes	Jack mackerel	*Trachurus japonicus*	Extract	-	-	
Cichliformes	Tilapia	*Oreochromis sp.*	Extract	-	Ore m 4	
Istiophoriformes	Swordfish	*Xiphias gladius*	Extract	-	Xip g 1	
Lutjaniformes	Red snapper	*Lutjanus campechanus*	Extract	-	-	
Perciformes	Grouper	*Epinephelus sp*	Extract	-	-	
	Barramundi	*Lates calcarifer*	-	-	Lat c 1	Lat c 1
					Lat c 6	
	Walleye pike	*Sander vitreus*	Extract	-		

Table 1. (Continued)

			Commercial IgE-test*		WHO/IUIS allergens	CRD-approach**
	Redfish	*Sebastes marinus*	-	-	Seb m 1	
	Perch	*Perca sp.*	Extract	-		
Pleuronectiformes	Plaice	*Pleuronectes platessa*	Extract	-	-	
	Halibut	*Hippoglossus hippoglossus*	Extract	-	-	
	Whiff	*Lepidorhombus whiffiagonis*	Extract	-	Lep w 1	
	Sole	*Solea solea*	Extract	-	-	
	Gulf flounder	*Paralichthys albigutta*	Extract	-	-	
Scombriformes	Tuna	*Thunnus albacares*	Extract	-	Thu a 1 Thu a 2 Thu a 3	Thu a 1
	Chub mackerel	*Scomber japonicus*	Extract	-	-	
	Mackerel	*Scomber scombrus*	Extract	-	Sco s 1	Sco s 1
	Indian mackerel	*Rastrelliger kanagurta*	-	-	Ras k 1	
Trachichthyiformes	Orange roughy	*Hoplostethus atlanticus*	Extract	-	-	

*Commercial systems (Phadia ImmunoCAP; Siemens Immulite; Hitachi Optigen CLA); **component-resolved diagnosis (CRD), according to current state of knowledge; 1, parvalbumin; 2, enolase; 3, aldolase; 4, tropomyosin; 6, collagen

long time as a hallmark of fish allergy. Studies of the recent decade revealed more differentiated insights into reactivity profiles.

Food challenge-based studies on fish cross-reactivity are rare. Previous studies reported on high levels of cross-reactivity related to IgE testing and clinical history [25, 26]. Several studies recorded data based on questionnaires. A Dutch study (total, $n = 38$) reported that 59% of the fish-allergic patients had an allergy to all fish species ever tried [27]. A Japanese study (total, $n = 38$) referred high cross-reactivity in 88% of the participants [28]. A recent study (total, $n = 35$) analyzed clinical cross-reactivity in a double-blind placebo-controlled food challenge (DBPCFC) design [23••]. According to objective symptom scoring, 43% of the participants reacted to all studied fishes while 54% tolerated at least one fish (subjective symptoms scoring: 68% non-tolerant, 29% partially tolerant). Thus, the overall prevalence of patients with broad and limited cross-reactivity might be estimated at 70% and 30%, respectively (Fig. 1). Another study (total, $n = 18$) revealed cross-reactivity among bony fishes and in most patients, tolerance to ray [29•]. Food allergy to a single fish has been reported in case studies (e.g. cod, salmon, sole, swordfish, catfish and conger fish) [30–35] and limited cross-reactivity to tuna/marlin and pangasius/tilapia [36, 37]. The

percentage of patients with monoreactivity to salmonids (salmon, trout) was 12% in another European cohort (total, n = 62) [38].

Medical diagnosis

As for other food allergies, the DBPCFC is the golden standard in fish allergy diagnosis. Low symptoms-eliciting doses have been reported (e.g. cod ED10, 0.7 mg and 23.8 mg for subjective and objective symptoms, respectively) [23••, 39]. Food challenges are usually not performed as the testing is extensive and with inherent health risk for the patient. Diagnostic mainstays are the careful record of the clinical history and IgE tests. Direct IgE tests include serum titration using fish extracts and optionally, fish parvalbumin from carp or cod (Table 1). Skin reactivity testing using fish or commercial fish extracts can be done in addition. Upon established diagnosis, the clinical management relies on a strict avoidance diet and medication of adverse symptoms [40].

Fish is one of the food allergens that need to be labeled mandatorily on all products irrespective of the percentage in the food [41]. This legislation has been implemented in order to contribute to the better safety of allergic patients. Still, this does not prevent accidental fish intake.

Occupational fish allergy

The occupational environment of a fish-processing working place entails important levels of exposure to allergens [42, 43]. A great load with airborne fish proteins (up to 986 $ng.m^{-3}$) including allergens has been measured for the fish-processing environment [44]. Cooks have high exposure rates by skin contact, inhalation but also ingestion [3]. Cutaneous symptoms (contact urticaria, protein contact dermatitis), allergic rhinitis and asthma are the most common symptoms. Preceding atopy and hand eczema are risk factors for fish sensitization via the damaged skin barrier [45]. The current diagnostic work-up includes sera IgE testing and skin tests (prick, prick-to-prick) with diagnostic fish extracts or the native food [3].

Fish allergens

Parvalbumins

The major fish allergen is parvalbumin [46]. This acidic muscle protein (10–12 kDa) occurs in all fish even though molecular characteristics vary across the species. Parvalbumin belongs to the family of divalent ion-binding 'EF-hand' proteins. They are involved in cellular ion homeostasis and muscle relaxation. Fish parvalbumin has two functional motifs ('EF-hand motifs') binding calcium- or magnesium-ions. The apo-protein has a lower IgE-binding capacity as compared with the ion-charged molecule, concluding that important IgE epitopes are located in the ion-binding regions [47]. Parvalbumin mutants with modified EF-hand motifs feature reduced IgE-binding capacity and low stability to gastrointestinal digestion

[48, 49]. Otherwise, parvalbumin has great molecular stability under thermal, chemical and proteolytic conditions.

Parvalbumins are clustered into the α- and the β-lineage [6, 46]. The β-lineage has a lower isoelectric point (pI < 4.8) compared with the α-lineage. Bony fish muscle contains mostly β-parvalbumin while α-parvalbumin is expressed by cartilaginous fish (e.g. rays and sharks). Most bony fish express several allergenic parvalbumins. The allergome database comprises 257 allergenic parvalbumins from 230 fish species (www.allergome.org accessed on 2019-07-16). Parvalbumins approved by the Allergen Nomenclature World Health Organization (WHO)/International Union of Immunological Societies (IUIS) Sub-Committee are summarized in Table 1.

Various studies showed high IgE prevalence to parvalbumins (> 70%) in fish-allergic patients, such as in European patients, as well as patients with reactivity to Asia-Pacific fish species [23••, 38, 50, 51]. Cross-reactive parvalbumin B cell epitopes are located in highly conserved protein regions, especially at the ion-binding sites [47]. Also, molecule-specific epitope regions are known, such as for salmonid fish parvalbumins, that explain limited cross-reactivity to those species [30, 31]. IgE cross-inhibition testing may reveal the patient's primary sensitization by determination of the most potent inhibitor protein [29•, 38]. Levels of IgE cross-reactivity vary, with high cross-recognition among parvalbumins from closely related fish and low cross-recognition in distantly related fish [52, 53]. That way, low IgE cross-recognition of ray α-parvalbumin was found in patients with primary sensitization to the bony fish homologue β-parvalbumin [29•]. This correlated with the patients' clinical reactivity as most tolerated ray. A subgroup of parvalbumin-positive patients has IgE antibodies recognizing not only fish β-allergen but also α-homologs from different meats (frog, chicken, crocodile) (Table 2) [53–56]. Unexpected clinical cross-reactions are known to occur in those patients.

Parvalbumin is found in less amount in dark fish muscle whereas light fish muscle contains high levels. Fish with light muscle tissue, such as cod or carp, have a high parvalbumin content of ca. 2.5–5.0 mg allergen/g muscle tissue while the allergen is mostly not detectable in dark muscle fish (e.g. tuna and

Table 2. Cross-reactive food to bony fish, extracts available in commercial sera IgE assays, approved WHO/IUIS allergens and molecule identity

			Commercial IgE-test	WHO/IUIS allergens	Component
Rajiformes	Ray	*Raja clavata*	-	-	α-parvalbumin
Galliformes	Chicken	*Gallus domesticus*	Extract	Gal d 8	α-parvalbumin
				Gal d 9	
				Gal d 10	
Crocodylia	Crocodile	*Crocodylus porposus*	-	Cro p 1	β-parvalbumin
				Cro p 2	α-parvalbumin
Anura	Edible frog	*Rana esculenta*	-	Ran e 1	α-parvalbumin
				Ran e 2	β-parvalbumin

swordfish) [57, 58]. Insights on parvalbumin contents are of translational relevance [59]. Low parvalbumin content fish are often tolerated, essentially in those patients with parvalbumin-specific sensitization only.
Food processes like heating and canning can result in parvalbumin epitope modification and thus, finally affecting IgE recognition [60, 61]. Parvalbumins can oligomerize or even form high molecular weight states, entailing also important changes in IgE binding [62]. Overall, fish canning seems to reduce IgE binding.

Enolases and aldolases

These muscle proteins are minor fish allergens. Both glycolytic enzymes occur in different isoforms. They belong to the family of 'TIM barrel proteins' characterized by a conserved tertiary protein folding of a barrel-like structure. Enolase binds magnesium-ions. Enolase (50 kDa) and aldolase (40 kDa) were identified as fish allergens in cod, salmon and tuna (Table 1) but also blunt snout bream and Nile perch [38, 63–65]. The prevalence of IgE binding seems to vary greatly for different fishes. In a previous study, 56% of the patients had IgE antibodies to cod enolase but only 19% to tuna enolase, 37% to cod aldolase but only 13% IgE to tuna aldolase [38]. Higher prevalence of cod-specific IgE in comparison with salmon and mackerel homologues was confirmed recently [23••]. Co-sensitization to cod parvalbumin, enolase and aldolase was concluded to correlate to severity of the clinical reaction to this fish [23••]. Both fish enolase and aldolase seem to be cross-reactive (with high inter-individual variation), although to a lower extent as compared with parvalbumins. Both enolase and aldolase are labile to thermal treatment [29•, 38].

Collagen and gelatin

Collagen and gelatin are minor fish allergens in European patients. Fish collagen is a highly stable protein mainly found in the skin, bone and other connective tissue [66]. This large molecule (300–400 kDa) is composed of three, helix-twisted chains. Glycine, alanine, proline and hydroxyproline are the major amino acids present in the primary structure. Gelatin is derived from fibrous collagen, by acidic or alkaline treatment, resulting in a water-soluble peptide mix.
First, IgE reactivity to tuna collagen was detected in fish-allergic patients from Japan [67]. Severe anaphylaxis was reported also in a clinical case with fish gelatin hidden in sweets [68]. The IgE prevalence for fish gelatin was found 19.3% in 62 fish-allergic individuals from Europe [38]. Later, IgE reactivity against mackerel collagen was confirmed for Japanese patients (50%; total, n = 34), including demonstration of effector cell reactivity triggered by the food allergen and cross-reactivity between homologs from 22 different fish species. IgE cross-reactivity appears to be limited for collagens from bony and cartilaginous fish [69–71]. So far, two fish collagens have been approved as official allergens, homologues from Atlantic salmon (Sal s 6) and barramundi (Lat c 6) (Table 1). Fish collagen has to be declared on food products in the USA but in Europe, it is exempted from mandatory labeling [40].

Other fish allergens

Fish tropomyosin has been identified in Mozambique tilapia as a 32 kDa allergen (Ore m 4) [72]. Recently, IgE reactivity to fish tropomyosin has been reported in another study [73]. Various other IgE-binding fish proteins have been described. Their clinical relevance is not yet fully understood. Ingestion of fish roe may also lead to allergic sensitization and clinical allergy. The allergen Onc k 5 (chum salmon) is vitellogenin, a 118 kDa protein and precursor of yolk proteins [74]. Roe allergens from different fish species are cross-reactive but there is no cross-reactivity to hen's egg or fish muscle allergens.

Occupational fish allergens

Parvalbumins are important allergens in the fish-processing work environment where sensitization occurs via the skin or the respiratory tract. Other high molecular weight compounds seem to play also a role in patients with allergic rhinitis and asthma. Beyond parvalbumin, further allergens have been described for clinical case, including phaseolin, creatine kinase and α-actinin-3 [75–78]. Their diagnostic relevance is still unclear.

Component-resolved diagnosis: why and when

Diagnostic extracts

The current IgE-based diagnosis of fish-allergic patients (serum, skin) is based on the use of fish extracts (Table 1). Diagnostic food allergen extracts are known to be variable in content [5••]. These variations depend mainly on the food source used for protein extraction as well as the extraction protocol. The parvalbumin content in diagnostic skin extracts was reported to vary from 20 to 70 μg/ml of extract (no parvalbumin detectable in single samples) while the protein content ranged from 320 to 2270 μg/ml extract [57]. Thus, the ratio of parvalbumin to total protein varied greatly from 1 to 13.4%. Recently, diagnostic extracts were evaluated by antibody-based testing, in combination with a proteomics approach and IgE-binding analysis [79]. Distinct variations (up to factor 10) were found when comparing samples from different providers and fish species, in respect to total protein, allergen content and IgE recognition patterns. Many samples did not contain the complete spectrum of relevant allergens, including parvalbumin, enolase, aldolase and collagen suggesting diagnostic imprecision in the skin test when using the diagnostic extract.

Diagnostic IgE testing

Singleplex testing is currently available for quantification of IgE binding to a number of extracts (n = 28) as well as 2 allergens, recombinant parvalbumins from carp and cod (Table 1). Given the fact that hundreds of fish species are available on the market, it becomes clear that not complete

spectra can be targeted but the use of representative fish extracts from relevant taxonomic orders.

Component-resolved testing, comparing a single allergen vs fish extracts, has been reported in several studies, mostly in the context of allergen identification. However, studies beyond IgE-binding measurement but integration of IgE data with food challenge data are scarce.

A single DBPCFC trial (total, $n = 35$) correlated so far the clinical reactivity to different fish (cod, salmon, mackerel) with IgE reactivity to extracts and single fish allergens, parvalbumin, enolase and aldolase, from the respective species [23••]. High diagnostic sensitivity was found for both IgE tests with fish extracts and parvalbumins. Extract-specific IgE (cod, salmon) discriminated best between individuals reacting to all fish vs those reacting to single/specific fish only, concluding that patients with particular IgE titers (cod, > 8.2 kU_A/L; salmon, > 5 kU_A/L) shall be advised to avoid any fish. Parvalbumins were confirmed as markers for clinical cross-reactivity [23••, 80]. However, they showed poor ability to identify patients with partial tolerance. Specific IgE to enolase and aldolase, together with anti-parvalbumin IgE, were concluded to be markers for more severe clinical reactions. This translational study demonstrated that both reagents are needed, fish extracts as well as the single molecules.

Other studies described that fish-allergic patients might have specific IgE, recognizing only the parvalbumin from specific fish, correlating with their clinical mono-sensitivity. Patients with salmonid fish allergy had IgE to salmon and trout parvalbumin fish only [30, 31]. Similarly, parvalbumin-specific IgE corresponding to species-specific clinical profiles were confirmed by others [33].

In a systematic approach, a panel of parvalbumins covering homologues from representative taxonomic groups might be advisable, in addition to the respective fish extracts. Such a parvalbumin selection is proposed in Table 1, amended by cod enolase and aldolase. Both cod and carp parvalbumin might not be required as they are highly cross-reactive [81]. Other fish allergens might be covered by the extracts but ideally, shall be included as further diagnostic molecules (e.g. collagen). Based on the variety of proposed diagnostic molecules, fish allergy is a showcase for future multiplex IgE testing in the format of in-vitro diagnostic platforms (e.g. ALEX, Macro Array Diagnostics; ImmunoCAP ISAC, Thermo Scientific). Finally, it is important to point out that the current state of knowledge on diagnostically relevant fish allergens is based on limited patients' cohorts that are characterized by specific patterns of fish consumption and local diets.

Diagnostic challenges and research needs

Avoid or not?

Fish-allergic patients feature various levels of cross-reactivity (Fig. 1). Most react to many fish, sometimes with severe reactions, and shall be advised to avoid any type of fish [24]. A parvalbumin-positive IgE test, together with

confirmation from the clinical history and possibly, skin tests with fresh fish, may address the diagnosis of those individuals.
Other patients have partial tolerances to specific fish and unnecessary avoidance of all fish should be prevented [23••]. However, a precise in-vitro diagnosis remains challenging in those cases. Parvalbumin-specific IgE tests are often false positive. Mostly allergens other than parvalbumins might play a role. Once the tolerated species is/are identified and the patient starts to introduce it/those in the diet, the question is whether the established tolerance is sustainable or whether the food allergy might spread to the tolerated species as well. Reference literature data on longitudinal patient follow-up upon reintroduction, including IgE-pattern development, are missing here.

Which fish?

Patients with parvalbumin-specific IgE only often tolerate low-parvalbumin fish such as tuna or swordfish [57, 58]. Systematic searches for further low-parvalbumin fish are lacking—they might reveal new dietary alternatives for patients with moderate to high threshold dose reactivity. Species distantly related to bony fish, such as cartilaginous fish (e.g. ray) expressing α-parvalbumins, might be consumed safely but this needs further clinical confirmation [29•]. For patients with suspected outgrown fish allergy, fish reintroduction schemes (from low to high allergenic fish) would be useful, together with corresponding specific IgE markers, to minimize food allergy recurrence and thus, to support the clinical management and patient follow-up. As an important practical note, when the consumption of specific fish is targeted, attention needs to be payed to the food product as species mislabeling does occur on the market [82].

Challenging basophils with fish?

The basophil activation test (BAT) emerged as a useful test in food allergy diagnosis, with similar sensitivity but superior specificity as compared with IgE tests (serum, skin) [83•]. Recently, BAT has been reported as being of importance to identify oral tolerance to ray in fish-allergic patients [29•]. Further studies on the clinical validation of BAT will be required in order to establish the diagnostic performance of this cellular assay in fish-allergic patients. Recommended study conditions include food challenge-proven allergy and the comparative testing of several allergens.

Conclusion

Fish-allergic patients are diverse in many ways, the severity of the clinical reaction including variable threshold doses, symptoms-eliciting species and the level of clinical cross-reactivity. Many fish allergens have been identified in the past decades contributing to a better understanding of the clinical reactivity profiles. Parvalbumin-specific IgE are markers for cross-reactions. Co-

sensitization to parvalbumin and enolase/aldolase refers to severity of the clinical reaction. Fish extracts still represent an indispensable basis for in-vitro diagnosis. As such, molecular fish allergy diagnosis represents a showcase for in-vitro multiplex approaches with the overarching goal to avoid unnecessary food restrictions and thus, improve patient care.

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Compliance with ethical standards

Conflict of interest
Julia Klueber declares that she has no conflicts of interest. Denise Schrama declares that she has no conflicts of interest. Pedro Rodrigues declares that he has no conflicts of interest. Heinrich Dickel declares that he has no conflicts of interest. Annette Kuehn declares that she has no conflicts of interest.

Human and animal rights and informed consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
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