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Study of the cross-reactivity of fish allergens based on a questionnaire and blood testing

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ELISA enzyme-linked immunosorbent assay
PBS phosphate buffered saline
PBST PBS containing 0.05% Tween 20

ABSTRACT

Background: Parvalbumin and collagen have been identified as cross-reactive allergens for fish allergies. Although doctors realize that various fish elicit allergies, the targets of food allergen labeling laws were only mackerels and salmons in Japan and mackerels in South Korea. This study aimed to reveal the causative species for fish allergy via questionnaires and blood tests.
Methods: Questionnaire research was conducted in Japan via the internet concerning allergies for fish-allergic patients or their family members. Next, IgE reactivities and cross-reactivities of 26 fish species were analyzed using sera obtained from 16 Japanese patients who were allergic to fish parvalbumin or collagen by enzyme-linked immunosorbent assay (ELISA) and inhibition ELISA.
Results: Questionnaire research revealed that 88% patients cannot eat mackerel and salmon in addition to other fish. In addition, 85% respondents were not satisfied with the current food allergen labeling law. In ELISA analyses, we clarified that pooled serum obtained from patients with fish parvalbumin-specific allergies exhibited IgE reactivity to the extracts of most fish species, and pooled serum obtained from patients with fish collagen-specific allergies displayed IgE reactivity to the extracts of all types of fish. Inhibition ELISA experiments revealed cross-reactivities of parvalbumin or collagen to extracts from all fish tested.
Conclusions: Most patients with fish allergies displayed allergic symptoms following the intake of various fish species. In addition, fish parvalbumin and collagen were causative factors of fish allergy and were highly cross-reactive fish panallergens. Therefore, current laws should be revised in Japan and South Korea.

Introduction

Fish is a valuable source of proteins, physiologically active substances such as eicosapentaenoic acid and docosahexaenoic acid, and minerals such as calcium. Thus, fish plays an important role in human nutrition and health. In parallel with the increase of fish consumption, however, fish allergy mediated by IgE has become a serious problem worldwide, especially in coastal countries such as Japan.

Extensive studies using the Baltic cod (*Gadus callarias*) first identified parvalbumin (called Gad c 1), a sarcoplasmic Ca^{2+}-binding protein of approximately 10 kDa, as a fish allergen.[1,2] Subsequent molecular studies revealed that parvalbumin is the major fish allergen in a number of fish, such as carp (*Cyprinus carpio*),[3] Atlantic salmon (*Salmo salar*),[4] Japanese jack mackerel (*Trachurus japonicus*),[5] crimson sea bream (*Evynnis japonica*),[6] Pacific mackerel (*Scomber japonicus*),[7] and bigeye tuna (*Thunnus obesus*).[8] Parvalbumin is produced only in vertebrates, particularly at high concentrations in fish and amphibian muscles.[9] In addition, parvalbumin is known as a major allergen, with IgE-positive rates of 67%–100%.[7,10,11] It has been revealed that various fish have common allergenicity (cross-reactivity) via parvalbumins.[12–17]

In the early 2000s, type I collagen was identified as a new second allergen for fish allergy, and approximately 30% of Japanese patients with fish allergies appear to be sensitive to fish collagens.[18,19] Collagen is a connective tissue protein, which is present in the muscles, skin, and bones in large quantities. Collagen from several species of fish has been determined to be an allergen, and

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collagens from these types of fish have cross-reactivities with each other.[20]

In Japan, with revisions to the Food Sanitation Act, the Labeling System for Foods Containing Allergens was implemented for food products in 2002. Seven ingredients must be labeled on the packages of food products as specific ingredients, and 20 ingredients should be labeled as subspecific ingredients. As per this law, only mackerels and salmons are regarded as subspecific ingredients. Moreover, the only salmon species targeted for labeling is anadromous salmon, whereas landlocked salmons are not included in the labeling. However, anadromous salmons have the same genes as landlocked salmons, and it has been reported that the allergenicity of both types of salmons is similar.[21] Furthermore, doctors realize that patients who are allergic to mackerels and/or salmons react to various species of fish. As mentioned previously, patients with fish allergies seem to exhibit allergic symptoms in response to several types of fish via cross-reactivities of parvalbumin and/or collagen.

Although several hundreds of fish species are consumed in Japan, there is no report in which the cross-reactivities of multiple fish species were investigated simultaneously in relation to both parvalbumins and collagens. Therefore, this study aimed to reveal the causative species of fish allergy via the questionnaire research. Moreover, we investigated the allergenicity and cross-reactivity of parvalbumin and collagen using 26 types of fish that are commonly consumed in Japan.

Methods

Questionnaire research

Questionnaire research was anonymously conducted for fish-allergic patients or their family members through the internet. The implementation period was from May 2014 to July 2015. In total, 97 responses to the questionnaire, including 95 valid responses, were received.

Samples

The following 26 species of fish, which are widely consumed in Japan, were used as samples: Round herring (*Etrumeus teres*), Pacific herring (*Clupea pallasii*), Japanese sardine (*Sardinops melanostictus*), chum salmon (*Oncorhynchus keta*), silver salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*S. salar*), Pacific cod (*Gadus macrocephalus*), splendid alfonsino (*Beryx splendens*), Mediterranean flying fish (*Cheilopogon heterurus*), Pacific saury (*Cololabis saira*), goldeye rockfish (*Sebastes thompsoni*), rosy seabass (*Doederleinia berycoides*), Japanese amberjack (*Seriola quinqueradiata*), Japanese jack mackerel (*T. japonicus*), chicken grunt (*Parapristipoma trilineatum*), red seabream (*Pagrus major*), white croaker (*Pennahia argentata*), swordfish (*Xiphias gladius*), red barracuda (*Sphyraena pinguis*), skipjack (*Katsuwonus pelamis*), Japanese Spanish mackerel (*Scomberomorus niphonius*), Pacific mackerel (*S. japonicus*), blue mackerel (*Scomber australasicus*), bigeye tuna (*T. obesus*), and yellowfin tuna (*Thunnus albacares*). Fresh filet samples (for 10 species of fish, including chum salmon, silver salmon, Atlantic salmon, Pacific cod, Japanese amberjack, swordfish, skipjack, Japanese Spanish mackerel, bigeye tuna, and yellowfin tuna) or round fish samples (for the remaining 16 species of fish) were purchased from local retail shops in Tokyo and immediately subjected to experiments. Round herring, chum salmon, white croaker, and bigeye tuna were used only for the investigation of parvalbumin, and Pacific herring, Pacific cod, Japanese amberjack, and Japanese Spanish mackerel were used only for the investigation of collagen. In addition to fish, third-stage larvae of *Anisakis simplex* with cysts were collected from the hepatopancreas surface of the Alaska pollack (*Gadus chalcogrammus*). After the cysts were digested with pepsin according to the method of Bier *et al.*,[22] the larvae were washed with a 0.9% NaCl solution and stored at −20 °C until use.

Preparation of extracts

In case of fillets, skin, bones, and dark muscles were removed from the fillets. Only white muscles were individually minced well, and a part of each minced sample was used for experiments. In case of round fish, the head, fins, skin, bones, whole organs, and dark muscles were removed, and only whole white muscles of each fish were used as described above for the experiments. For the analysis of parvalbumin, the following extract was used: the mince of white muscle collected from each specimen was homogenized in four volumes of 150 mM NaCl-10 mM phosphate buffer [pH 7.0; phosphate buffered saline (PBS)]. After heating at 100 °C for 10 min, the homogenate was centrifuged at 16,000 ×*g* for 5 min, and the obtained supernatant was used for experiments. The extracts were stored at −20 °C until further use. For the analysis of collagen, the following extract was used: the mince of white muscle collected from each specimen was homogenized in four volumes of 50 mM Tris-HCl (pH 8.0) containing 7 M urea and 2 M thiourea. The homogenate was centrifuged at 16,000 ×*g* for 5 min, and the obtained supernatant was used for experiments. The extracts were diluted 10-fold with the same buffer and immediately subjected to experiments without freezing.

For the preparation of an extract of *A. simplex*, the larvae were extracted with four volumes of PBS. After centrifugation at 16,000 ×*g* for 5 min, the supernatant obtained was used as a crude extract. The extract was stored at −20 °C until use.

Purification of parvalbumin and collagen

Parvalbumin was purified from the white muscle of Pacific mackerel according to the method of Shiomi *et al.*[8] Collagen was purified from the skin of Pacific mackerel according to the method of Miller and Rhodes.[23] The concentration of each allergen was determined according to the method of Lowry *et al.*[24] using bovine serum albumin as a standard.

Human sera

Sera were obtained from 16 fish-allergic patients with documented clinical histories of immediate hypersensitivity reactions after the ingestion of fish. All the patients were Japanese. Written informed consent was obtained from each patient, and patient anonymity was preserved. First, all patients were checked by medical doctors and were diagnosed to be allergic to fish. Next, all patients were tested using ImmunoCAP (Phadia, Uppsala, Sweden), and the classes for some fish extracts were two to six. In addition to this, skin prick testing was done, and all the patients gave positive results for various fish species. IgE reactivities of patients' sera to Pacific mackerel parvalbumin or collagen were checked by enzyme-linked immunosorbent assay (ELISA). Sera from patients with parvalbumin-specific allergy ($n = 8$) and collagen-specific allergy ($n = 8$) were used for the following experiments. In the present study, pooled serum from 10 healthy volunteers (Cosmo Bio, Tokyo, Japan) was used as a control. All experiments using human sera were performed following the Ethical Guidelines of Tokyo University of Marine Science and Technology (Permit Number: 26-002). The study was conducted in accordance with the principles embodied in the Declaration of Helsinki.

ELISA and inhibition ELISA

Wells of a microtiter plate (Nunc MaxiSorp; Thermo Fisher Scientific, Hudson, NH, USA) was coated with 50 μl of the purified Pacific mackerel parvalbumin, purified Pacific mackerel collagen, *A. simplex* extract, or fish extracts (1 μg/ml purified allergens or extracts of fish meats or *A. simplex*, diluted 1:1,000 with 25 mM carbonate buffer, pH 9.5) at 37 °C for 2 h. After washing four times with 400 μl of PBS containing 0.05% Tween 20 (PBST), the plate was blocked with 300 μl of 20% Blocking One solution (Nacalai Tesque, Kyoto, Japan) at 4 °C overnight. After washing with PBST, the plate was reacted with 50 μl of human serum (diluted 1:50 with PBST containing 5% Blocking One) at 37 °C for 2 h. Then, the plate was washed with PBST and reacted with 50 μl of horseradish peroxidase-conjugated goat anti-human IgE antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) solution (400 ng/ml in PBST containing 5% Blocking One) at 37 °C for 1 h. After washing with PBST, the plate was incubated with 50 μl of substrate solution (ELISA POD Substrate TMB Kit, Popular; Nacalai Tesque) at 37 °C for 10 min. The enzyme reaction was terminated by the addition of 50 μl of 1 M sulfuric acid, and the developed color was analyzed by the absorbance at 450 nm using a microplate reader.

The cross-reactivities of parvalbumin or collagens from Pacific mackerel to extracts from 26 species of fish were examined via ELISA inhibition experiments. Each patient's serum was diluted 1:25 with PBST containing 5% Blocking One and incubated at 37 °C for 1 h with an equal volume of the purified parvalbumin or collagen solution (20 μg/ml) in PBST containing 5% Blocking One. A 50-μl aliquot of this solution was then added to a microtiter plate that had been previously coated with extracts from the 26 aforementioned species of fish described above. The subsequent procedure was the same as that for the ELISA described above.

All ELISAs and inhibition ELISAs were performed in triplicate, and the data are expressed as mean values.

Results

Summary of patient answers to the questionnaire

A summary of the patient answers to the questionnaire is shown in Table 1. In total, 66% of the respondents were identified as fish-allergic patients, whereas the remaining respondents were family members of patients with fish allergy. The subjects consisted of 53 males and 42 females. The proportion of patients aged 1–5 and 6–11 years old were 32% and 25%, respectively, compared with 13%, 14%, and 7% for those in their 20s, 30s, and 40s, respectively. Less than 4% of the respondents were not aged 1–11 and 20–49 years. Seventy two percent of patients were diagnosed with fish allergy by medical doctors, and 28% of patients were self-diagnosed or diagnosed by a family member.

Frequency and symptoms of fish allergy according to the questionnaire

The frequencies and symptoms of fish allergy are shown in Table 2. When asked, “Does the patient always experience an allergy when he or she eats fish,” 48% and 32% of patients or their family members answered “Always experience an allergy” and “Some fish causes allergy whereas others do not,” respectively. No other response was provided by more than 9% of the respondents.

Twenty-eight percent of patients were not diagnosed with a fish allergy by medical doctors (Table 1). When asked “Does the patient always experience an allergy when he or she eats fish?,” 41% of them (12% of patients) answered “Always experience an allergy. (Table 2)” They were expected to be truly patients with fish allergies. Therefore, at least 84% of patients [patients diagnosed by a doctor (72%) plus patients who always experience an allergy without a diagnosis by medical doctors (12%)] were expected not to be allergic to *A. simplex*, which infects sea fish and can cause allergy, or not to have experienced histamine poisoning (scombroid fish poisoning). A possibility cannot be denied that the remaining 17% of patients experienced an allergic reaction to *A. simplex* or histamine poisoning.

The symptoms experienced by patients included urticaria (62%), itching of skin (61%), oral allergy syndrome (52%), and erythema (33%). Respiratory symptoms (suffocation, asthma, and/or dyspnea) were observed in 32% patients. Approximately 16%–20% of patients had experienced gastrointestinal symptoms, such as abdominal pain, vomiting and Diarrhea. Other symptoms were reported by less than 15% of the respondents.

Answers related to causative fish, fish products, and the allergen labeling regulation

The fish species that cause allergy are presented in Table 3. In total, 42% and 34% of the respondents answered “(Probably) all

Table 1
Characteristics of the study patients.

		n	%
Patient	Self	63	66.3
	Family member	32	33.7
Sex	Male	53	55.8
	Female	42	44.2
Age	0 year old	0	0.0
	1–5 years old	30	31.6
	6–11 year old	24	25.3
	12–19 year old	3	3.2
	20's	12	12.6
	30's	13	13.7
	40's	7	7.4
	50's	3	3.2
	60's	2	2.1
	70's	1	1.1
	Over 80 years old	0	0.0
Diagnosed by doctor	Yes	68	71.6
	No	27	28.4

Table 2
Frequencies and symptoms of fish allergy.

Question/answer	n	%
Does the patient always experience an allergy when he or she eats fish?		
Always experience an allergy	46	48.4
Experience when physical condition is poor	6	6.3
Can usually eat fish but sometimes experience allergy	1	1.1
Experience allergy if fish is not fresh	4	4.2
Some fish causes allergy whereas others do not	30	31.6
Others	8	8.4
What symptoms does the patient experience when he/she eats fish? (multiple answers allowed)		
Urticaria	59	62.1
Erythema	31	32.6
Itching of skin	58	61.1
Stomach pain	9	9.5
Abdominal pain	19	20.0
Vomiting	20	21.1
Diarrhea	15	15.8
Oral allergy syndrome	49	51.6
Injected eye	6	6.3
Sneezing, runny nose and/or nasal congestion	14	14.7
Suffocation, asthma and/or dyspnea	30	31.6
Hypotension	9	9.5
Others	0	0.0

Table 3
Questionnaire about causative fish species, fish products, and allergen labeling regulation.

Question/answer	n	%
Which type of fish is the patient allergic to?		
(Probably) all kinds of fish	40	42.1
Eel	4	4.2
Sardine	18	18.9
Salmon	20	21.1
Rainbow trout	4	4.2
Cod	15	15.8
Saury	12	12.6
Horse mackerel	23	24.2
Swordfish	7	7.4
Skipjack	7	7.4
Mackerel	22	23.2
Tuna	10	10.5
Others	32	33.7
Recount of the above results		
(Probably) all kinds of fish	40	42.1
Cannot eat fish other than mackerel/salmon if not all	44	46.3
Only mackerel and/or salmon	11	11.6
Can patient eat fish if it is cooked?		
Cannot eat fish in any cases	58	61.1
Can eat well-cooked fish although I cannot eat raw fish	12	12.6
Others	25	26.3
Can patient eat fish-paste products, such as Japanese *kamaboko*, *chikuwa*, *hanpen*, *satsuma-age*, *tsumire*?		
Can eat	45	47.4
Cannot eat	33	34.7
Others	17	17.9
Mackerel and salmon should be labeled on packages of processed food products. Do you think that all types of fish should be labeled on the packages besides mackerel and salmon?		
Yes, all types of fish should be labeled	81	85.3
No, labeling for mackerel and salmon is sufficient	9	9.5
Others	5	5.3

kinds of fish" without listing each fish and "Others," respectively. These respondents were more numerous than those who listed individual types of fish. Patients who reacted to mackerel and salmon were 23% and 21%, respectively. Patients who reacted to other fish species were as follows: horse mackerel, 24%; sardine, 19%; cod, 16%; saury, 13%; and tuna, 11%. Less than 8% of patients reacted to other species. When these answers were recounted, it was found that only 12% of patients reacted to only mackerel and/or salmon, and the remaining patients could not eat different types of fish. Forty-five percent of patients who react to only mackerel and/or salmon were self-diagnosed or diagnosed by a family member to have fish allergy.

Regarding whether patients can eat cooked fish, 61% of patients responded that they could not eat fish under any circumstances. When asked, "Can the patient eat fish-paste products, such as Japanese *kamaboko*, *chikuwa*, *hanpen*, *satsuma-age*, and *tsumire*," 47% of patients answered affirmatively.

In response to the question, "Mackerel and salmon should be labeled on packages of processed food products. Do you think that all types of fish should be labeled on the packages besides mackerel and salmon," 85% of patients and family members of patients answered, "Yes," which greatly exceeded the 10% of who answered, "No."

ELISA

Sera obtained from 16 patients with fish allergies and from healthy control donors were checked for IgE reactivity to the purified Pacific mackerel parvalbumin and collagen and to the extract of *A. simplex* (Fig. 1). Eight sera (Patients #1–8) obtained from the patients with fish allergies showed IgE reactivity to the purified Pacific mackerel parvalbumin but did not react with the purified Pacific mackerel collagen and the extract of *A. simplex*. The remaining eight patients (Patients #9–16) had elevated IgE levels to the purified Pacific mackerel collagen, but their sera did not react with the purified Pacific mackerel parvalbumin and the extract of *A. simplex*. In addition, the pooled control serum did not react with any of the samples. Therefore, the sera from the former eight patients that were monosensitized to parvalbumin were pooled in equal amounts and were used for parvalbumin experiments. Sera from the latter eight patients with fish collagen-specific allergies were pooled in equal amounts and were used for collagen experiments. There was no possibility that the patients' sera reacted with the *A. simplex* extract.

The IgE reactivities of patients with fish parvalbumin-specific allergies against extracts from 22 species of fish are shown in Figure 2A. IgE reactivities varied with fish species. Highly intensive IgE reactivities were observed for round herring, splendid alfonsino, Mediterranean flying fish, goldeye rockfish, rosy seabass, Japanese jack mackerel, and red barracuda. In contrast, patients with parvalbumin-specific allergies did not react to extracts from chum salmon, silver salmon, swordfish, skipjack, bigeye tuna, and

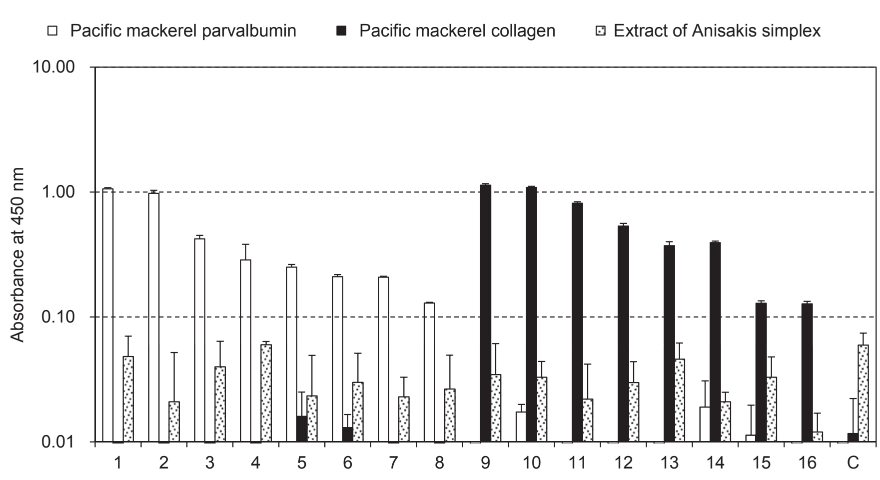

Fig. 1. Enzyme-linked immunosorbent assay (ELISA) of IgE reactivity to the purified Pacific mackerel parvalbumin, purified Pacific mackerel collagen, and crude extract of *A. simplex* in the sera from 16 patients. C indicates the pooled sera obtained from 10 healthy controls. The data are expressed as mean + SD.

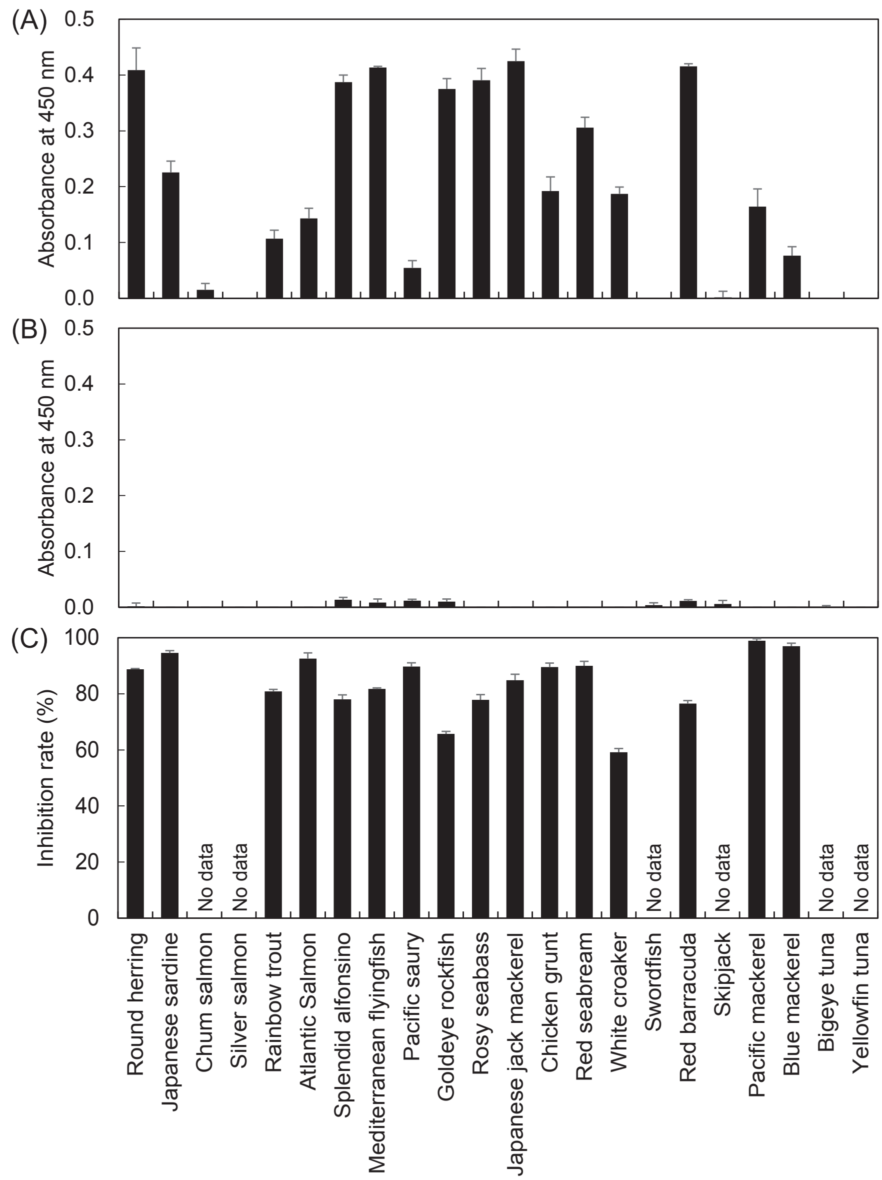

Fig. 2. IgE reactivities of the pooled sera from patients with parvalbumin-specific allergies to the extracts of 22 fish species. Pooled sera from patients (**A**) and healthy controls (**B**) were subjected to enzyme-linked immunosorbent assay. Inhibition of IgE reactivities of the pooled patient sera to extracts of 16 fish species by purified Pacific mackerel parvalbumin (**C**). The data are expressed as mean + SD.

yellowfin tuna. The pooled control serum did not react with extracts from any species of fish (Fig. 2B).

Inhibition ELISA was performed using the pooled serum from patients with parvalbumin-specific allergies (Fig. 2C). The reactivities of extracts from 22 types of fish were inhibited by purified Pacific mackerel parvalbumin. However, six fish species that did not react to the pooled patient serum were eliminated from inhibition ELISA. In case of white croaker, the inhibition rate was 59%. High inhibition rates (66%–99%) were recorded for the remaining species, and cross-reactivities via parvalbumin were observed among all 16 types of fish.

Figure 3A shows the IgE reactivities of extracts from 22 types of fish as assessed by ELISA using pooled serum obtained from eight patients with collagen-specific allergies. Unlike in the case of parvalbumin, the pooled serum from these patients reacted with extracts of all 22 species of fish. The following five fish exhibited strong IgE reactivities: rainbow trout, Atlantic salmon, skipjack, Japanese Spanish mackerel, and yellowfin tuna. The IgE reactivities of extracts from Pacific cod, splendid alfonsino, and rosy seabass were slightly lower than those for other types of fish. The absorbance of the pooled control serum was less than 0.1 for all fish and was considered to be IgE-negative (Fig. 3B).

Inhibition ELISA was performed using the pooled serum obtained from eight patients with fish collagen-specific allergies (Fig. 3C). The reactivities of the extracts from 22 types of fish were inhibited by purified Pacific mackerel collagen. The inhibition rates of Pacific cod and splendid alfonsino were 48% and 57%, respectively, and the remaining fish species displayed high inhibition rates (63–83%). Therefore, cross-reactivities were observed between Pacific mackerel collagen and extracts from all 22 species of fish.

Discussion

In this study, we revealed the causative species of fish allergies based on questionnaire research and confirmed the IgE reactivities

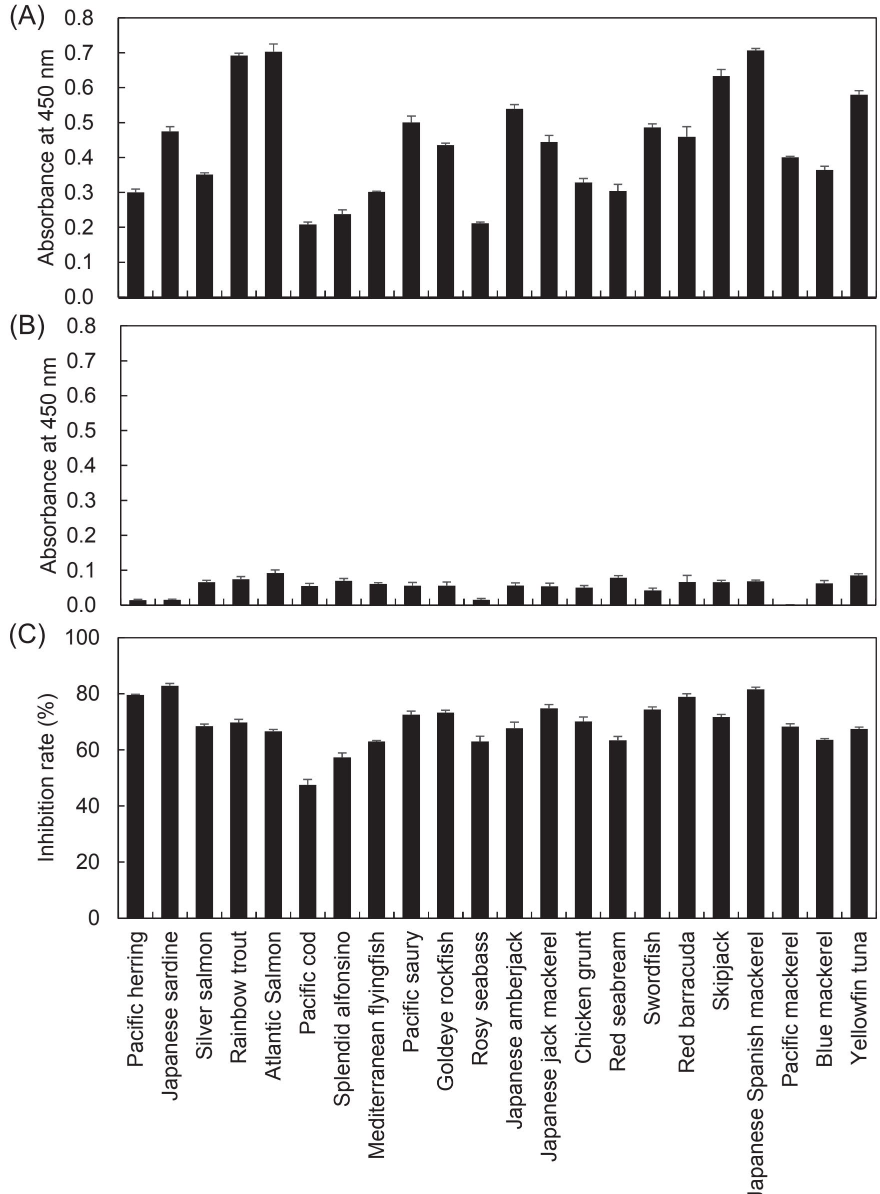

Fig. 3. IgE reactivities of the pooled sera from patients with fish collagen-specific allergies to the extracts of 22 fish species. Pooled sera from patients (**A**) and healthy controls (**B**) were subjected to enzyme-linked immunosorbent assay. Inhibition of the IgE reactivities of the pooled patient sera to extracts of 22 fish species by purified Pacific mackerel collagen (**C**). The data are expressed as mean + SD.

and cross-reactivities of various fish by ELISA and inhibition ELISA using patient sera. According to a survey conducted by the Ministry of Health & Welfare in Japan, the tendency to have fish allergies increases as people age. However, the patient population in this study consisted primarily of infants, elementary school students, and probably their parents because questionnaire research was rolled out mainly via an organization of fathers who have children with allergies (Association of Fathers of Kids with Food Allergies). Therefore, the potential number of adult patients with fish allergies could be higher.

In our study, it was revealed that many patients with fish allergies constantly experience allergic symptoms following the intake of fish. Concerning the allergic symptoms, urticaria, itching of skin, and oral allergy syndrome were commonly reported. However, the proportion of patients with respiratory symptoms, which tend to be severe, was comparatively high at 32%. Therefore, the significance of fish allergy should be noted.

Regarding fish, only mackerels and salmons are labeled on packages by the food allergen labeling law against processed foods in Japan. Probably because the survey conducted by the Ministry of Health & Welfare in Japan demanded answers about particular fish, it appears that the causative fish species were limited to only a few fish species. Our research revealed that approximately 12% patients react only to mackerel and/or salmon. It was revealed that approximately 90% of the respondents are not satisfied with current allergen labeling regulation, and most patients with fish allergies are sensitive to various fish species.

There is a possibility that 17% of patients experienced an allergic reaction to *A. simplex* or histamine poisoning. Approximately half of the patients who react to only mackerel and/or salmon were self-diagnosed or diagnosed by a family member to have fish allergy. Mackerel is the major cause of histamine poisoning among fishes. In addition, salmon is a representative fish causing *A. simplex* allergy.

Many reports demonstrated that parvalbumins from various fish exhibited IgE reactivities and cross-reactiveties.[12–17] Meanwhile, IgE reactivities and cross-reactivities have been confirmed to collagens from five types of fish, including Japanese eel, splendid alfonsino, Pacific mackerel, skipjack, and bigeye tuna.[20] Regarding the results of ELISA using sera obtained from patients with fish parvalbumin-specific allergies, the IgE reactivities of extracts from 22 species of fish widely varied. These patients did not react or slightly reacted to extracts of mackerel and salmon. In contrast, ELISA using sera obtained from patients with fish collagen-specific allergies indicated that extracts from all 22 types of fish displayed IgE reactivities without exception. Interestingly, extracts of rainbow trout, Atlantic salmon, skipjack, and bigeye tuna, to which patients with fish parvalbumin-specific allergies reacted weakly, were strongly recognized by the sera of patients with fish collagen-specific allergies. In addition, all species of fish that we tested displayed cross-reactivities with Pacific mackerel collagen, indicating that fish collagen is a fish panallergen in addition to parvalbumin.

It is known that the allergenicity of parvalbumin is highly thermostable.[1,25,26] Collagen also has thermostable IgE reactivities.[27] Our research revealed that majority of fish-allergic patients cannot eat fish even when it is cooked. However, the allergenicity of Japanese fish-paste products is known to be low.[28] This is attributed to a water-bleaching effect through which water-dissolved parvalbumin is removed from fish meats.[28] Our survey revealed that 47% of fish-allergic patients can eat Japanese fish-paste products. Therefore, fish-paste products are promising as hypoallergenic foods for patients with fish parvalbumin-specific allergies.

Although only 26 types of fish were analyzed, this study demonstrated that parvalbumin and collagen are panallergens for fish allergy. Therefore, all fish species may elicit allergic reactions in fish-allergic patients. Although collagen seems to exhibit IgE reactivities in all species of fish, sera obtained from patients with fish parvalbumin-specific allergies did not react with the extracts of some fish species. The concentration of parvalbumin in muscles significantly varies among fish, and the allergenicity of fish in patients with fish parvalbumin-specific allergies depends on the concentration of parvalbumin.[15,29,30] If the concentration of parvalbumin in the meats of many fish species and causative allergens can be easily identified in hospitals, doctors can provide dietary instructions for patients with fish parvalbumin-specific allergies.

Regarding fish, the targets of food allergen labeling regulations are only mackerels and salmons in Japan and mackerels in South Korea. However, this study highlighted the risk of allergic reactions among many fish-allergic patients. Therefore, it is imperative that the current food allergen labeling laws should be revised in Japan and South Korea.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

YK managed all research, conducted experiments, and wrote this manuscript. JH conducted the experiments. SI conducted the questionnaire research. NH-S contributed to manuscript revision. All authors have read and approved the final manuscript.

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