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ORIGINAL ARTICLE

Grades of 43 Fish Species in Japan Based on IgE-binding Activity

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ABSTRACT

Background: Hypersensitivity reactions to fish are a common food allergy, but IgE-binding activity to fish species have not been fully elucidated. The aim of this study was to identify fish with high binding activity to IgE in sera from Japanese fish-hypersensitive individuals.

Methods: 38 children with a history of at least one episode of hypersensitivity after ingestion of fish were enrolled and 34 children with no history of reactions and negative IgE results for at least five kinds of fish antigen were included as controls. Using a radioallergosorbent test, we examined IgE-binding to each fish species using sera from fish-hypersensitive subjects. Fish were then graded according to IgE-binding activity.

Results: Many fish species, including red salmon, silver salmon, yellowfin tuna, big eyed tuna, Atlantic tuna, saurel, skipper, yellowtail, Japanese sardine, bonita and mackerel had high IgE-binding activity. All of these fish are abundantly consumed in Japan. The hypersensitivity reactions experienced by many subjects occurred after ingestion of species with high IgE-binding activity. Only halibut (Osteichthyes) and sharks (Chondrichthyes) had low IgE-binding activity.

Conclusions: A correlation was observed between IgE levels and expression of symptoms after fish ingestion. High consumption of salmon, tuna, scad (including saurel), skipper, yellowtail, sardine, bonita and mackerel in Japan might be the cause of the high IgE-binding activity of these species. The grades of fish species consumed widely in Japan are likely to be useful for nutritional instruction of fish-allergic patients.

KEY WORDS
classification, fish, grade, IgE binding, Z-score

INTRODUCTION

Many kinds of seafood such as fish and crustaceans are abundantly consumed in Japan as well as in Southern and Northern Europe, and consumption is also increasing in the United States.[1] Fish and processed fish products are important sources of highly assimilated animal protein, as well as n-3 polyunsaturated fatty acid and fat-soluble vitamins such as vitamin D.

It was recently suggested that a reduced ratio of n-3 and n-6 fatty acids might be responsible for the development of diseases such as thrombosis[2] and allergic diseases.[3] Many physicians therefore recommend increased consumption of fish high in eicosapentaenoic acid.

In Northern and Southern Europe, the high prevalence of fish allergies is a serious issue. However, in Japan, despite the high consumption of fish, there are limited reports on fish allergies. According to a nationwide questionnaire carried out in Japan in 1999,[3] 10.6% of pediatric patients with food hypersensitivities were allergic to seafood. This prevalence was preceded by allergies to egg and dairy products. However, despite the above, we do not know which fish are likely to result in fish-hypersensitivity in Japan. In the present study, we therefore graded fish species consumed commonly in Japan based on the tertiles of IgE *z*-scores.

METHODS

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Table 1 Patient list

	Age	Gender	Complication	History of hypersenstive reactions to fish ingestion	Total serum IgE (IU/ml)
1	6m	M	AD, BA	saurel: E	1770
2	6m	M	AD	sardine: E/halibit: U	430
3	7m	M	AD	cod・sardine・salmon: U	734
4	8m	F	AD	brown sole: U	ND
5	8m	F	AD	sardine・black rockfish・mackerel・brown sole: U	105
6	9m	M	AD	brown sole・flounder: U	ND
7	10m	M	AD, BA	perch: U/porgy: E	940
8	11m	M	AD	brown sole・blue grenadier・tuna: E, U	3040
9	1y0m	M	AD, BA	brown sole: E	2060
10	1y1m	M	AD	big eyedtuna・silver salmon・genuine porgy: E, I	ND
11	1y2m	F	AD	atoka mackerel: U	1090
12	1y3m	M	AD	hake: U	217
13	1y5m	F	AD, BA	red eel goby: U	12
14	1y6m	M	AD	yellowtail・skipper・sardine・saurel: U, V	546
15	1y6m	M	AD, BA	porgy: E/file fish・saurel・brown sole・barracuda・eel・silver salmon: U	840
16	1y11m	M	AD	salmon・porgy: E	16452
17	2y2m	F	AD, BA	black rockfish・greenling: E	11000
18	2y3m	M	AD	perch・atoka mackerel: U/red eel goby: W	79
19	2y3m	F	AD	salmon・saurel: E	ND
20	2y5m	M	AD	yellowtail・conger eel: E/saurel: U	4550
21	2y7m	M	AD	salmon: I/tuna: U	680
22	2y8m	M	AD, BA	cod: E/sardine・sillago: I	540
23	2y10m	M	AD	cod: E	4010
24	3y4m	M	AD	tuna: U/yellowtail: D	203
25	3y5m	M	AD, BA	salmon・tuna: W, E	5250
26	3y6m	F	AD	red eel goby: U, V	470
27	3y6m	M	AD	big eyed tuna・genuine porgy: U	ND
28	3y8m	M	AD	saurel・sardine: E	1069
29	3y9m	F	AD, BA	eel・saurel: R/salmon・sardine・cod・tuna: U	535
30	4y7m	M	AD	brown sole・rainbow trout・tuna・salmon: R, U	5730
31	4y7m	M	AD	conger eel: U/sardine: E, I	1190
32	5y9m	M	AD	mackerel: U/black rockfish・pink cusk-eel: I/hake: I, E	7000
33	6y4m	F	AD	saurel: E	1070
34	7y4m	M	AD, BA	eel: U, RD	1120
35	7y9m	M	AD	porgy・flounder・brown sole・salmon・tuna・sardine: E, I, N/tuna: E, I	5570
36	9y4m	F	AD	tuna: OAS	2698
37	10y0m	F	AD	whiting: OAS/mackerel: U/red salmon: I	4196
38	11y8m	F	AD	red salmon: E, I, P	ND

AD; atopic dermatitis, BA; bronchial asthma
erythema (E), urticaria (U), itching (I), lip swelling (S), dyspnea (Dysp), wheezing (W), diarrhea (Dia), vomiting (V), nauzea (N), oral tingle (T), abdominal pain (P)
ND: not done

SUBJECTS

38 pediatric subjects (males : females = 26 : 12 ; mean age ± SD = 3y2 m ± 2y10 m; range: 6 months to 11 years) with fish allergies were enrolled in this study (Table 1). They were diagnosed as having fish allergies based on at least one convincing episode of hypersensitive reaction after fish ingestion and positive results (more than class 2) to at least one fish-specific IgE using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden).

All subjects had atopic dermatitis, and 10 of 38 (26.3%) subjects had bronchial asthma. All had experienced at least one episode of clinical allergic reactivity to one or more fish species. Skin symptoms such as urticaria, itching and reddishness frequently occurred in 37 of 38 subjects. Respiratory symptoms oc-

Table 2 Fish species tested and biological classification and Grades of fish species according to the teritiles of the IgE z-score in patients

Class	Order	Family	Genus	Species	Common name	Z-score mean (SD)	L	M	H (†)
Osteichthyes	*Clupeiformes*	*Clupeidae*	*Clupea*	*Clupea pallasii*	herring	2.77 (2.45)		○	
			Sardinops	*Sardinops melanostictus*	japanese sardine	3.91 (3.09)			○
		Engraulidae	*Engraulis*	*Engraulis japonicus*	japanese anchovy	2.69 (2.75)		○	
	Anguilliformes	*Anguilla Schranjk*	*Anguilla*	*Anguills japonica*	eel	3.75 (3.34)			○
				Anguilla anguillae	eel	3.42 (2.69)			○
		Congridae	*Conger*	*Conger myriaster*	conger eel	2.97 (2.75)		○	
	Salmoniformes	*Osmeridae*	*Hypomesus*	*Hypomesus trarspacificus nipponensis*	pond smelt	2.55 (3.11)		○	
			Spirinchus	*Spirinchus lanceolatus*	shishamo smelt	3.74 (3.56)			○
		Salmonidae	*Oncorhynchus*	*Oncorhyncus nerka nerka*	red salmon	3.12 (2.50)			○
				Oncorhynchus kisutch	silver salmon	3.88 (3.03)			○
				Oncorhynchus mykiss	rainbow trout	3.84 (3.03)			○
		Pleciglossidae	*Plecoglossus*	*Plecoglossus altivelis*	ayu	3.62 (3.00)			○
	Beloniformes	*Scomberesocidae*	*Cololabis*	*Cololabis saira*	skipper	3.18 (2.53)			○
		Exocoetidae	*Prognichthys*	*Cypselurus agoo agoo*	flying fish	3.34 (2.93)			○
	Gadiformes	*Gadidae*	*Theragra*	*Gadus macrocephalus*	cod	2.69 (2.44)		○	
			Gadus	*Theragra chalcogramma*	whiting	4.43 (3.57)			○
		Merluccidae	*Merluccius*	*Merluccius*	hake	3.79 (3.27)			○
			Macruronus	*Macruonus novaezalan-diae*	blue grenadier	3.05 (2.67)			○
	Lophiformes	*Lophiidae*	*Lophiomus*	*Lophiomus setigerus*	angler	3.05 (2.68)			○
	Perciformes	*Sphyraenidae*	*Sphyraena*	*Sphyraena japonica*	japanese barracuda	3.67 (2.82)			○
		Carangidae	*Seriola*	*Seriola dumerili*	amberjack	2.58 (2.47)		○	
				Seriola quinqueradiata	yellowtail	3.27 (3.32)			○
			Trauchurus	*Trachurus japonicus*	saurel	4.06 (3.48)			○
		Percichthyidae	*Lateolabrax*	*Lateolabrax japonicus*	perch	3.04 (2.36)			○
		Pomadasyidae	*Parapristipoma*	*Parapristipoma trilineatum*	chicken grunt	3.60 (2.71)			○
		Sillaginidae	*Sillago*	*Sillago japonica*	sillago	3.15 (2.48)			○
		Sparidae	*Pagrus*	*Pagrus major*	genuine porgy	2.96 (2.64)		○	
		Scombridae	*Euthynnus*	*Katsuwonus pelamis*	bonita	3.35 (2.90)			○
			Scomber	*Scomber japonicus*	mackerel	4.17 (3.45)			○
				Scomber scombrus	mackerel	3.08 (2.60)			○
			Thunnus	*Thunnus albacares*	yellowfin tuna	3.05 (3.11)			○
				Thunnus obesus	big eyed tuna	3.89 (3.25)			○
				Thunnus thynnus	atlantic tuna	3.46 (2.55)			○
		Trichiuridae	*Trichiurus*	*Trichiurus japonicus*	snakefish	2.91 (2.12)		○	
		Sciaenidae	*Pennahia*	*Pennahia argentata*	white croaker	3.76 (2.66)			○
	Scorpaeniformes	*Hexagrammidae*	*Pleurogrammusl*	*Pleurogrammus azonus*	atka mackerel	2.75 (2.41)		○	
	Pleuronectiformes	*Paralichthyidae*	*Paralichthys*	*Paralichthys olivaceus*	flounder	2.86 (2.35)		○	
		Pleuronectidae	*Hippoglossus*	*Hippoglossus stenolepis*	halibut	1.86 (1.95)	○		
			Limanda	*Pleuronectes herzensteini*	brown sole	2.54 (2.55)		○	
chondrichthyes	*Carcharhiniformes*	*Carcharhinidae*	*Prionace*	*Prionance glauca*	blue shark	1.34 (2.33)	○		
		Triakididae	*Mustelus*	*Mustelus manazo*	smoothhounds	2.61 (2.01)		○	
				Mustelus griseus	spotless smooth-hounds	1.79 (2.28)	○		
	Lamniformes	*Lamnidae*	*Lamna*	*Lamna ditropis*	salmon shark	1.63 (2.75)	○		

(†) tertiles of the IgE z-score
L: Low-tertile
M: Middle-tertile
H: High-tertile

curred in 3 of 38 (7.9%) subjects and gastrointestinal symptoms developed in 5 of 38 (13.2%). Total IgE levels were 2662 ± 3563 IU/ml (mean ± SD). Thirty-four children (males: females = 21 : 13; mean age ± SD = 4

Table 3 The order of fishes in the ranking based on number of patients with episode of hypersensitive reactions

	Fish	Number of patients
1	salmon	12
2	sardine, scad (saurel) , tuna	9
3	brown sole (brown flounder, fluke)	8
4	porgy	6
5	cod, mackerel	4
6	black rockfish, eel, red eel goby, yellow-tail	3
7	atka mackerel, conger eel, hake	2

Table 4 The order of fishes in the ranking based on the annual consumption quantity per family in Japan (2001)

	Fish	Quantity per family (*g*)
1	salmon	5293
2	tuna	3443
3	scad (saurel)	3397
4	skipper	2147
5	yellowtail	2026
6	sardine	1984
7	bonita	1713
8	mackerel	1519
9	brown flounder	1485
10	porgy	730

Table 5 Two groups of patients at positive RAST ratio

groups	Patient proportion
positive for a few species (1 − 3)	5/38 (13%)
positive for multiple species (4 − 43)	33/38 (87%)

y8 m ± 3y6 m; range: 3 m to 11y9 m) with no histories of allergic reactions to fish and negative results (class 0) to at least five fish-specific IgE were enrolled as control subjects. Serum samples were collected from all subjects and stored at −20℃ until use.

PREPARATION OF FISH EXTRACTS

43 fish species, 39 Osteichthyes (bony fish) species (9 orders, 24 families, 31 genera) and 4 Chondrichthyes (sharks) species as shown in Table 2. Fish species were classified according to "Fishes of Japan with Pictorial Keys to the Species".[4] The Osteichthyes species include fishes commonly consumed in Japan.

Fish meat extracts were obtained from Toyo Suisan Marine Industry Co. then minced, raw or frozen, with a speed cutter (Matsushita Industry Company, Tokyo, Japan). Five grams of each meat sample were vortexed in 15 ml of 1M KCl-PBS in sterile centrifuge tubes then placed overnight in a cold room (4℃). After addition of a further five ml of1M KCl-PBS, samples were centrifuged at 13,000 rpm. The supernatants were dialyzed against distilled water then concentrated by dialysis with a filter (cut off: 6,000−8,000 of the molecular weight) under high pressure for 1−2 days in a cold room. The concentrates were lyophilized and stored at −20℃.

MEASUREMENT OF SPECIFIC IgE ANTIBODIES TO FISH MEAT EXTRACTS USING RADIOALLERGOSORBENT TEST (RAST)

Freeze-dried samples (dry weight: 75 mg) were dissolved with 25 ml of coupling buffer (0.1M $NaHCO_3$, 0.5M NaCl) and centrifuged at 13,000 rpm. Cyanogen bromide-activated paper disks were then soaked in each extract solution and incubated with rotation at 4℃. After aspirating the supernatant, 25 ml of blocking buffer (0.2M glycine buffer) was added to the disks then incubated with rotation at room temperature for 5 hours. The disks were then washed with 0.1M acetate buffer and blocking buffer alternately 5 times then once with PBS-Tween before suspension in 40 ml of PBS-Tween and stored in a cold room until use (4℃).

For analysis, the fish extract-conjugated paper disks were incubated for 5 hours with 25 µl of the subjects' serum and 25 µl of PBS-Tween then, after washing with PBS-Tween, 25 µl of ^{125}I-labeled anti-IgE (IgE-RIABEAD, DAINABOT Co., Ltd., Tokyo, Japan), approximately 2,200 Bq, and 25 µl of PBS-Tween were added before incubating overnight. After free radioisotopes were removed by rinsing with PBS-Tween, bound radioisotopes were measured in a gamma counter. Results were expressed as the percent binding of total radioactivity added.

Specific IgE levels were determined using RAST then log transformed for statistical analysis. To standardize the values in each survey, they were converted to *z*-scores as previously described.[5,6] The *z*-score represents the number of standard deviations by which a subject's IgE level differs from the mean of the control group, with logarithms of the serum specific IgE level used to normalize the distribution. Z-scores of specific IgE antibody titers (percent binding) were calculated with each fish species using the following formula: *z*-score = (A-B)/C where A = the % IgE binding of the subject, B = the mean % IgE binding of the controls and C = the standard deviation of the % IgE binding of controls. We used *z*-score to compare with IgE binding activity between each fish species.

STATISTICAL ANALYSIS

Non-parametric analysis of variance (Mann-Whitney *U*-test) was used to determine the significance of variance between groups with a p value of less than 0.05 was considered statistically significant. Statistical analyses were performed utilizing Statview version 4.5 (Avacus Concepts, Inc., Berkeley, USA).

RESULTS

43 fish species were graded based on IgE z-scores of 38 fish-allergic subjects. Table 2 shows the fish species tested and their biological classification, and grades according to the tertiles of IgE z-score. IgE z-scores ranged from 1.34 ± 2.33 to 4.43 ± 3.57 (mean ± SD) and three grades, tertiles,[4,5] were determined as follows: low (IgE z-score <2), middle (IgE z-score ≥ 2 <3), and high (IgE z-score ≥ 3). Differences between groups with regard to IgE z-score were significant ($p < 0.0001$). Whiting (*Theragra chalcogramma*) had the highest IgE z-score followed by mackerel (*Scomber japonicus*), saurel (*Trachurus japonicus*), Japanese sardine (*Sardinops melanostictus*), big eyed tuna (*Thunnus obesus*), and silver salmon (*Oncorhynchus kisutch*), to name but a few of the 27 species in the high tertile group. On the other hand, blue shark (*Prionance glauca*), salmon shark (*Lamna ditropis*), spotless smoothhounds (*Mustelus griseus*) and halibit (*Hippoglossus stenolepis*) had low tertile IgE scores. Sharks (Chondrichthyes) therefore showed relatively low IgE-binding activity in Japanese subjects, while those listed in the high tertile group were all included in Osteichthyes. However, the grades of Osteichthyes species based on the tertiles of IgE z-score were not concordant with the biological classification (Table 2).

Table 3 shows the order of fish that were reported to elicit hypersensitive reactions in the subjects enrolled in this study. Of these, salmon, tuna, sardine, scad (including saurel), mackerel and eel were included in the high tertile group. In the 28 fish species which were reported to cause practical hypersensitive reactions for patient subjects, 17 species (60.7%) had high z-scores, 5 species (17.9%) had middle z-scores and 1 species (3.6%) had a low z-score. We did not measure IgE binding activity for the remaining 5 species.

According to the Statistics Bureau of the Ministry of Public Management, Home Affairs, Post and Telecommunication, Japan (2001), salmon, tuna, scad (including saurel), skipper, yellowtail, sardine, bonita, mackerel, brown flounder and porgy are abundantly consumed in Japan (Table 4). Of these, salmon, tuna, scad (including saurel), skipper, yellowtail, sardine, bonita and mackerel were shown to have high IgE-binding activity in Japan.

Subjects were also divided into 2 groups according to the number of fish species that tested positive for IgE binding (Table 5); IgE binding values above the mean +2 SD of the control were defined as positive. Five of the 38 patients (13%) tested positive for 1−3 species, while 33 (87%) tested positive for 4−43 species.

DISCUSSION

The IgE-binding activity of 43 fish species was measured with RAST using sera from 38 unconfirmed fish-allergic subjects with fish specific IgE. We compared IgE binding activity between each fish species. Whiting (*Theragra chalcogramma*), mackerel (*Scomber japonicus*), saurel (*Trachurus japonicus*), Japanese sardine (*Sardinops melanostictus*), big eyed tuna (*Thunnus obesus*) and silver salmon (*Oncorhynchus kisutch*), to name but a few, showed high tertile z-scores, while sharks and halibut showed low tertile z-scores; these fish are abundantly consumed in Japan. The results suggest that this high consumption might be responsible for the high IgE-binding activity of these species. Subjects were divided into two groups, one that tested positive for IgE binding to few fish (1−3 species) and another that tested positive to many fish (4−43 species), suggesting that species-specific allergens cause allergies to few fish, while common allergens cause allergies to many fish species.

A correlation was observed between IgE levels and expression of symptoms after fish ingestion. Bernhisel-Broadbent *et al.*[1] reported that the clinical reactivity to different fish species does not necessarily correspond to specific IgE antibody titers. More recently, the use of a quantitative method to measure food-specific IgE antibodies (CAP System FEIA) has been shown to be more predictive of symptomatic IgE-mediated food allergies.[7] The fish-specific IgE level indicating that an individual is more than 95% likely to have an allergic reaction if he or she ingests that specific fish is 20 kUa/L. However, there is considerable overlap in values between challenge-positive and -negative subjects with IgE levels below this cut-off titer. We therefore need to rank the allergenicity of different fish species based on the number of hypersensitive reactions observed after ingestion. Table 3 shows the order of fish that elicited hypersensitive reactions in the subjects enrolled in this study. There was a strong correlation between the ranking of fish based on IgE-binding activity and the number of hypersensitive episodes. Furthermore, we found that many fish species, which caused hypersensitivity reactions in subjects, had a high z-score. Therefore we could assume some relationship between IgE binding activity and allergenicity. On the other hand, another 5 species had middle z-scores and 1 species had a low z-score, suggesting that specific IgE levels are not necessarily consistent with expression of hypersensitivity reactions, as is the case with other food allergens. We did not perform a food challenge test with each fish species for each subject. Further study will be needed to elucidate the relationship between IgE binding activity to each fish species and allergenisity.

A relationship between IgE-binding activity and the fish species most commonly consumed in Japan was also observed. Mackerel, saurel, sardine, big eyed tuna, and silver salmon, which showed high tertile IgE z-scores, were among the top 10 fish species consumed in Japan.

Pascual *et al.*[8] previously assessed IgE titers specific to six fish species (hake (*Merluccius merluccius*), whiff (*Lepidorhombus whiffiagonis*), cod (*Gadus callarias*), witch (*Glyptocephalus cynoglossus*), sole (*Solea solea*), and Albacore (*Thunnus alaunga*)) and reported that hake, followed by whiff, is most likely to induce the greatest IgE response; both are frequently consumed in Spain. They suggested that the grade of IgE-binding activity might therefore be related to the degree of consumption. Our results support this conclusion.

Our subjects were divided into 2 groups according to the number of fish species that tested positive for IgE binding; one group tested positive for few species and the other for many species. Hansen *et al.*[9] demonstrated that fish contain both common allergens and species-specific allergens. Shared allergens such as parvalbumin are thought to be present in varying amounts in different species, thus contributing to differences in allergenic activity.[9] The presence of species-specific allergens, which are thought to be involved in differences in allergenic activity among fish species, has also been shown by Kelso *et al.*[10] who reported an exclusively swordfish-allergic patient from whom IgE antibodies reacted only to a swordfish protein of approximately 25 kD but not 13 kD. Bernhisel-Broadbent *et al.*[1] also showed the clinical allergenicity of different fish species using oral challenge tests. Thus, our results could be explained by cross-reactivity between fishes[9,11] or species-specific allergens. Interestingly, some fish-allergic subjects in the study developed hypersensitive reactions after ingestion of fish they had never before eaten. This might also be explained by cross-reactivity between fish or sensitization via the placenta or breast milk.

In this study, three shark species (blue shark, salmon shark and spotless smoothhounds) were graded as having low tertile IgE z-scores. One of the reasons for this finding might be that sharks are not commonly consumed in Japan. Sharks belong to the Chondrichthyes class whereas the other fish species tested in this study are species of Osteichthyes. In our preliminary study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that shark protein bands with a molecular mass of approximately 11 to 14 kD were more weakly stained with Coomassie brilliant blue than those of species of Osteichthyes (data not shown). These components were thought to represent parvalbumin analogous to cod Gad c1, a major allergen in cod. Parvalbumin commonly exists in fish meat and is responsible for cross-reactivity among fish meat allergens.[12] This might be another reason why the allergenicity of sharks is weaker than that of Osteichthyes fish species.

In conclusion, the findings in this study suggest that there is no specific relationship between IgE-binding activity and the biological classification of fish species of Osteichthyes and are supported by Kakami *et al.*.[13] The three grades determined for fish species consumed widely in Japan using the tertiles of IgE z-score provide us with useful information for nutritional instruction of fish-allergic patients. The number of subjects tested in this study was, however, limited and therefore a larger scale study based on clinical allergenicity is needed.

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