Original Article

Real-World Sensitization and Tolerance Pattern to Seafood in Fish-Allergic Individuals

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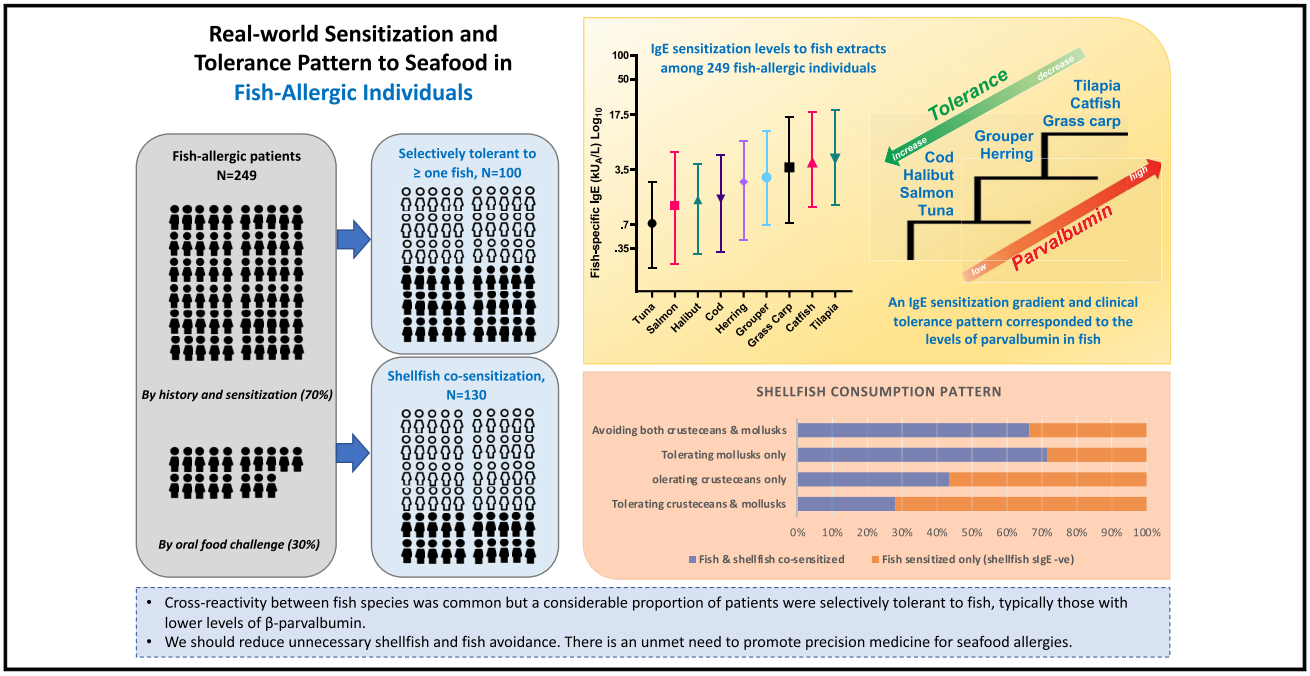
What is already known about this topic? Although significant cross-reactivity between fish species occurred, tolerance to selective fish has been reported but limited to small case series. Data on IgE sensitization and clinical reactivity to fish and shellfish are limited.

What does this article add to our knowledge? The article identified 40% of fish-allergic individuals being tolerant to selected fish, more commonly to fish with low β-parvalbumin levels. An IgE sensitization gradient corresponded to the β-parvalbumin levels of fish. Shellfish avoidance was common in non–shellfish-sensitized individuals, while IgE sensitization with clinical tolerance of shellfish was common.

How does this study impact current management guidelines? Our study provided evidence that fish-allergic individuals may exhibit selective tolerance toward specific types of fish, and accurately diagnosing shellfish allergies with conventional tests remains challenging. Precision medicine is necessary for seafood allergies.

VISUAL SUMMARY



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Abbreviations used
AD- Atopic dermatitis
CI- Confidence interval
DBPCFC- Double-blind placebo-controlled food challenge
D.p.- Dermatophagoides pteronyssinus
GC- Grass carp
IQR- Interquartile range
MWD- Mean wheal diameter
OFC- Oral food challenge
SCORAD- SCORing Atopic Dermatitis
sIgE- Specific IgE
SPT- Skin prick test

BACKGROUND: Seafood is a common cause of food allergy and anaphylaxis, but there are limited published real-world data describing the clinical presentation of fish and shellfish allergies.
OBJECTIVE: This study aimed to examine the clinical characteristics, immunological profile, and tolerance pattern to fish, crustaceans, and mollusks in fish-allergic individuals.
METHODS: Patients presenting with IgE-mediated fish allergy between 2016 and 2021 were recruited. A comprehensive sensitization profile including specific IgE and skin prick test to various fish and shellfish species and a detailed clinical history including individuals' recent seafood consumption were evaluated.
RESULTS: A total of 249 fish-allergic individuals (aged 4.2 ± 5.8 years) were recruited from 6 allergy clinics in Hong Kong, and they had experienced their fish-allergic reaction 2.2 ± 3.4 years before enrollment. Seventy-five subjects (30%) reacted to either grass carp, salmon, grouper, or cod in oral food challenges. We identified an IgE sensitization gradient that corresponded to the level of β-parvalbumin in fish. In total, 40% of fish-allergic individuals reported tolerance to 1 or more types of fish, more commonly to fish with a lower β-parvalbumin level such as tuna and salmon, compared with β-parvalbumin-rich fish such as catfish and grass carp. Despite fish and shellfish cosensitization, 41% of individuals reported tolerance to crustaceans, mollusks, or both, whereas shellfish avoidance occurred in half of the fish-allergic individuals, of whom 33% lacked shellfish sensitization.
CONCLUSIONS: Fish allergy commonly presents in early childhood. A considerable proportion of fish-allergic patients are selectively tolerant to certain fish, typically those with lower levels of β-parvalbumin. There is an unmet need to promote precision medicine for seafood allergies. © 2023 The Authors.

Key words: *Fish allergy; Sensitization; IgE; Tolerance; Consumption; Shellfish; Crustaceans; Mollusks; β-Parvalbumin; Fish allergenicity ladder*

Fish is a common cause of food allergy, particularly in coastal regions where fisheries supply is high.[1] Fish allergy tends to persist until adulthood[2,3] and is a known trigger of anaphylaxis in young children.[4] Because of the high degree of structural homology among parvalbumin isoforms across fish species,[5] cross-sensitization to fish species from other families occurs. It is also observed that in clinical practice, patients who have an allergic reaction to one fish often react to another fish,[6] and some even react with severe allergic reactions;[7] therefore, fish-allergic patients are often labeled as fish-allergic under one umbrella term and advised complete fish avoidance. Evidence on selective fish tolerance based on an oral food challenge (OFC) or consumption data was limited to studies with relatively small sample sizes.[8] The term "selective tolerance" describes individuals who are tolerant but to selected fish only.[6] Our group reported that 75% of our fish-allergic patients were "selectively tolerant" to salmon despite being allergic to grass carp (GC), as demonstrated by double-blind placebo-controlled food challenges (DBPCFCs).[9] Conversely, a proportion of European fish-allergic patients were found to react only to salmonids.[10] A recent study involving a multinational patient cohort reported that 21% of their fish-allergic patients displayed no IgE sensitization to at least 1 bony fish species.[11]

Physicians may conservatively advise a blanket approach in fish or shellfish avoidance when counseling patients and families with children allergic to fish or shellfish.[12] This approach is based on earlier findings that at least one-third of seafood-allergic individuals had allergic reactions to multiple seafood species.[13] It is also a common practice for families with fish-allergic children to avoid crustaceans like shrimp, crab, and lobster and mollusks like clam, scallop, oyster, mussels (bivalves), squid, cuttlefish (cephalopods), abalone, and snail (gastropod) for fear that their children may cross-react.[14] Such beliefs are fostered by the fact that "seafood" and "shellfish" in Chinese are often used interchangeably by the public.[15] Clinically, it is impractical to provide multiple OFCs for the diverse group of consumable fish and

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This work has been partly funded through the Health and Medical Research Fund (reference nos. 06170856, 08191356, 08191436, and 09202866), Hong Kong Research Grants Council (HKRGC) Early Career Scheme (reference no. 24109922), Research Impact Fund (reference no. R4035-19), Hong Kong Special Administrative Region Government, and TS Lo Foundation. The funders had no role in the development of the protocol, nor any role in the conduct of the data analysis or its publication.

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication October 10, 2022; revised September 2, 2023; accepted for publication September 26, 2023.

Available online ■■

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2213-2198

https://doi.org/10.1016/j.jaip.2023.09.038

shellfish species, especially in a resource-limiting setting.[16] Despite the lack of cross-reactivity between known allergens in crustaceans, mollusks, and fish,[17] epidemiological studies have shown that a proportion of fish-allergic individuals reported allergic reactions to shellfish or vice versa.[14,18] There were, however, limited data reporting the serological reactivity to shellfish in fish-allergic individuals.

There is an unmet need to develop more precise diagnostic strategies tailored for population-specific fish-allergic patients, to effectively assess patients with potential selective tolerance to fish in daily practice, and to give appropriate and safe clinical advice. We conducted a real-world study to evaluate the clinical characteristics, sensitization profiles, and tolerance patterns in fish-allergic individuals toward fish, crustaceans, and mollusks.

METHODS

Participants

The study population included 249 patients with a clinical history of fish allergy recruited from 6 hospitals in Hong Kong from July 2016 to December 2021. Fish allergy diagnosis was based on a history of immediate-type fish-allergic reactions and sensitization to fish either by skin prick test (SPT) and/or fish-specific IgE (sIgE). This study was reviewed and approved by the institutional review boards of the Joint Chinese University of Hong Kong—New Territories East Clinical Research Ethics Committee (Joint CUHK-NTE CREC), Kowloon Central/Kowloon East Cluster Research Ethics Committee (KC/KE CREC), Kowloon West Cluster Research Ethics Committee (KWC CREC), and the University of Hong Kong/Hospital Authority Hong- Kong West Cluster (HKU/HA HKW IRB), and written informed consents were obtained.

Allergy assessment

All participants underwent SPTs with a standard panel of fish mix (flounder, cod, and halibut), salmon, catfish, shellfish mix (crab, shrimp, lobster, and oyster), and *Dermatophagoides pteronyssinus* (*D.p.*) and *Dermatophagoides farinae* extracts (ALK-Abelló, Madrid, Spain). Saline and histamine phosphate 10 mg/mL were negative and positive controls, respectively. A result of the SPT was considered positive if the mean wheal diameter (MWD) was 3 mm or larger than the negative control. Participants' total IgE and sIgE levels to commercially available recombinant β-parvalbumins from Baltic cod (rGad c 1) and common carp (rCyp c1), as well as extracts of fish species that are frequently consumed in Asia,[19,20] including freshwater fish like catfish, GC, grouper, and tilapia and marine fish like cod, halibut, herring, salmon, and tuna, were tested using the ImmunoCAP platform (Thermo Fisher, Uppsala, Sweden) with the Phadia 200 analyzer according to the manufacturer's instructions. Results were considered positive if values were 0.35 kUA/L or above. In participants with voluntary consent, OFCs with GC (*Ctenopharyngodon idella*), salmon (*Salmo salar*), grouper (*Epinephelus spp.*), cod (*Gadus morhua*), and tiger prawn (*Penaeus monodon*) were performed as previously described.[9,21] Because no commercial extract was available for GC, which was the most popular fish consumed locally, additional SPTs with in-house raw and cooked GC extracts were arranged for individuals who returned for GC OFCs. The severity of atopic dermatitis (AD) was assessed in those who returned for OFCs. The article's Online Repository at www.jaci-inpractice.org provides a detailed methodology, including the methods to prepare the SPT GC extract.

Patients with physician-diagnosed fish allergies were interviewed during recruitment visits. Their clinical history and seafood consumption patterns after their initial reactions to fish were recorded using standardized forms. Participants were categorized as having "selective tolerance" to fish if they reported tolerance and regular consumption of 1 or more fish species within 2 years (Figure E1, available in this article's Online Repository at www.jaci-inpractice.org).

Participants were categorized as shellfish-sensitized if their SPT MWD to shellfish extract was 3 mm or greater and/or shellfish-sIgE titers were 0.35 kUA/L or greater, and as shrimp-allergic if they reacted at a DBPCFC to tiger prawn. Participants were further classified into the following groups: "avoiding both crustaceans and mollusks" if they had not consumed any crustaceans and mollusks, "tolerating crustaceans and mollusks" if both crustaceans and mollusks were consumed, "tolerating crustaceans only" if crustaceans but not mollusks were consumed, and "tolerating mollusks only" if mollusks but not crustaceans were consumed (Figure E2, available in this article's Online Repository at www.jaci-inpractice.org).

Statistical analyses

Quantitative variables were presented as mean with standard deviation or median with interquartile range (IQR), whereas qualitative variables were presented as number (percentage). Differences in baseline characteristics between participants with and without OFC results and between those with and without shellfish cosensitization were evaluated using the Pearson χ^2 test and the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables. For the comparison of the characteristics across participants with different shellfish consumption patterns, the Pearson χ^2 test for categorical variables and the nonparametric Kruskal-Wallis test and Dunn's multiple comparison *post hoc* test for fish and shellfish-sIgE levels were used. Pairwise comparison of parvalbumin levels was assessed using the Wilcoxon matched-pairs signed-rank test. The relationship between continuous variables was evaluated by the Pearson correlation coefficient. $P < .05$ was considered significant. Statistical analyses were performed using SPSS 25.0 (IBM Inc, Armonk, NY) and GraphPad Prism 7.0 (GraphPad Software Inc, San Diego, Calif).

RESULTS

Characteristics of fish-allergic participants

A total of 249 fish-allergic participants, with a median age of 4.2 years (IQR, 2.5-8.2 years), were enrolled in this study. Participants underwent SPT and blood tests 2.2 years (IQR, 1.1-4.5 years) after their initial reactions to fish. As shown in Table I, 164 (66%) participants were male, 234 (95%) had AD, 137 (56%) had allergic rhinitis, and 69 (28%) had asthma. A total of 143 (58%) participants reported additional food allergies other than fish, crustaceans, and mollusk allergies, and 102 (46%) carried adrenaline autoinjectors. The median age at the first fish-allergic reaction was 9 months (IQR, 7-12 months). Forty-seven subjects (38%) reported first allergic reactions to carp (GC and mud carp), 29 (23%) to salmon, and 21 (17%) to grouper, prepared by steaming and boiling in 107 (70%) and 37 (24%) allergic episodes, respectively. Angioedema was the most common presenting symptom in 170 (69%) of the individuals, followed by urticarial rash in 82 (33%). Overall, 40 (16%) participants fulfilled the diagnostic criteria for anaphylaxis.

We performed 111 positive OFCs in 75 subjects who were allergic to fish. The positive challenges included 54 (49%) with GC, 28 (25%) with salmon, 24 (22%) with cod, and 5 (5%)

TABLE I. Clinical characteristics and sensitization profile of fish-allergic participants

Characteristics	Total (N = 249)	Fish allergy based on history and sensitization (n = 174)	Fish allergy based on OFC results (n = 75)	*P* value*
Age of recruitment (y), median (IQR)	4.16 (2.47-8.31)	4.19 (2.33-8.53)	4.11 (2.85-7.44)	.743
Male, n (%)	164 (65.86)	112 (64.37)	52 (69.33)	.448
Asthma, n (%)	69 (28.05)	50 (29.24)	19 (25.33)	.530
Allergic rhinitis, n (%)	137 (55.69)	98 (57.30)	39 (52.00)	.440
Atopic dermatitis, n (%)†	234 (95.12)	161 (94.15)	73 (97.33)	.286
SCORAD, median (IQR)	n.a.	n.a.	14.40 (0.00-23.40)	n.a.
SCORAD index 0-25, n (%)	n.a.	n.a.	59/73 (80.82)	n.a.
SCORAD index 26-49, n (%)	n.a.	n.a.	9/73 (12.33)	n.a.
SCORAD index 50-74, n (%)	n.a.	n.a.	4/73 (5.48)	n.a.
SCORAD index 75-100, n (%)	n.a.	n.a.	1/73 (1.37)	n.a.
Chronic urticaria, n (%)	18 (7.32)	12 (7.02)	6 (8.00)	.785
Drug allergy, n (%)	9 (3.80)	7 (4.09)	2 (2.67)	.583
Parental atopy, n (%)	175 (86.21)	124 (87.94)	51 (82.26)	.279
Other food allergies, n (%)	143 (57.66)	99 (57.23)	44 (58.67)	.833
Own an AAInj, n (%)	107 (47.77)	70 (44.87)	37 (54.41)	.189
Allergic symptoms to fish, n (%)				
Angioedema	170 (68.83)	116 (67.44)	54 (72.00)	.477
Localized urticaria	82 (33.33)	57 (33.33)	25 (33.33)	1.000
Generalized urticaria	76 (30.77)	48 (27.90)	28 (37.33)	.140
Vomiting	38 (15.38)	32 (18.60)	6 (8.00)	**.034**
Dyspnea/wheeze/hoarseness	36 (14.57)	22 (12.79)	14 (18.67)	.229
Eczema flare, skin itchiness	71 (28.86)	49 (28.65)	22 (29.33)	.677
Anaphylaxis	40 (16.13)	24 (13.79)	16 (21.62)	.125
First fish allergic reaction				
Age (mo), median (IQR)	9.00 (7.00-12.00)	12.00 (7.00-12.00)	8.00 (6.00-10.00)	**.006**
First allergic fish, n (%)				.468
Carp	47 (18.88)	32 (18.39)	15 (20.00)	
Salmon	29 (11.65)	17 (9.77)	12 (16.00)	
Grouper	21 (8.43)	15 (8.62)	6 (8.00)	
Fish cooking method, n (%)				
Steamed	107 (42.97)	77 (44.25)	30 (40.00)	.637
Fried	8 (3.21)	7 (4.02)	1 (1.33)	
Boiled	37 (14.86)	25 (14.37)	12 (16.00)	
Age from 1st allergic reaction to recruitment (y), median (IQR)	2.21 (1.11-4.51)	2.16 (0.99-4.26)	2.68 (1.14-5.24)	.394
Skin prick test (SPT) result (mm), median (IQR)				
SPT to fish mix	4.50 (3.00-6.38)	4.00 (2.00-6.00)	5.00 (3.75-7.00)	**.003**
SPT to salmon	3.00 (0.00-5.00)	3.00 (0.00-4.50)	4.00 (2.00-6.13)	**.012**
SPT to catfish	5.50 (3.88-7.63)	5.00 (3.50-7.13)	6.50 (4.63-8.88)	**.004**
SPT to raw grass carp‡	n.a.	n.a.	7.75 (5.50-10.88)	n.a.
SPT to cooked grass carp‡	n.a.	n.a.	7.50 (5.50-9.50)	n.a.
Serological IgE (kUA/L), median (IQR)				
Specific IgE to tuna	0.73 (0.20-2.48)	0.68 (0.18-2.66)	0.83 (0.32-2.34)	.585
Specific IgE to salmon	1.23 (0.22-5.90)	1.02 (0.18-5.21)	1.99 (0.57-6.08)	.159
Specific IgE to halibut	1.46 (0.30-4.12)	1.31 (0.26-4.71)	1.68 (0.52-3.90)	.558
Specific IgE to cod	1.50 (0.32-5.41)	1.17 (0.27-5.37)	2.54 (0.68-5.50)	.116
Specific IgE to herring	2.47 (0.45-8.18)	1.76 (0.37-8.17)	3.96 (0.77-9.06)	.173
Specific IgE to grouper	2.81 (0.70-10.80)	2.58 (0.56-11.00)	3.93 (0.85-10.80)	.296
Specific IgE to grass carp	3.76 (0.74-16.50)	3.12 (0.55-17.00)	6.88 (1.71-16.33)	.061
Specific IgE to catfish	4.35 (1.19-18.80)	3.99 (0.82-22.50)	6.05 (1.56-17.60)	.422
Specific IgE to tilapia	4.88 (1.25-20.25)	4.49 (1.14-18.40)	7.30 (1.65-20.80)	.161
Specific IgE to rGad c 1	3.94 (0.78-14.48)	3.18 (0.72-14.30)	4.97 (1.19-14.70)	.389

(continued)

TABLE I. *(Continued)*

Characteristics	Total (N = 249)	Fish allergy based on history and sensitization (n = 174)	Fish allergy based on OFC results (n = 75)	*P* value*
Specific IgE to rCyp c 1	4.65 (0.95-22.38)	3.8 (0.87-25.10)	6.50 (1.66-16.10)	.387
Total IgE (kUA/L), median (IQR)	652.50 (303.75-1787.75)	660.50 (325.25-1794.75)	603.00 (229.00-1790.25)	.407
Subjects with reported fish tolerance, n (%)	100 (40.16)	80 (45.98)	20 (26.67)	**.004**
Fish with reported tolerance to, n (%)				
Salmon	71 (28.51)	58 (33.33)	13 (17.33)	**.010**
Tuna	23 (9.24)	19 (10.92)	4 (5.33)	.233
Halibut	20 (8.03)	17 (9.77)	3 (4.00)	.202
Cod	16 (6.43)	16 (9.20)	0 (0.00)	**.004**
Grouper	15 (6.02)	12 (6.90)	3 (4.00)	.563
Carp	12 (4.82)	12 (6.90)	0 (0.00)	**.020**
Catfish	6 (2.41)	5 (2.87)	1 (1.33)	.671
Herring	4 (1.61)	3 (1.72)	1 (1.33)	1.000
Tilapia	4 (1.61)	3 (1.72)	1 (1.33)	1.000

AAInj, Adrenaline autoinjector; *IQR*, interquartile range; *n.a.*, not applicable; *OFC*, oral food challenge; *SCORAD*, SCORing Atopic Dermatitis.
**P* value between individuals with fish allergy diagnosis based on history and sensitization and those with fish allergy diagnosis based on oral food challenge results. Significant values ($P < .05$) are bolded.
†Severity of atopic dermatitis, as defined by SCORAD, was assessed in individuals with fish allergy who underwent oral food challenges.
‡Skin prick test to in-house raw and cooked grass carp was only performed in participants who returned for oral food challenges to fish.

with grouper. Forty-seven (63%) subjects reacted to 1 fish, 23 (31%) to 2 fish, 6 (8%) to 3 fish, and none to 4 fish. When GC and salmon DBCPFCs were compared, GC, a β-parvalbumin-rich fish, had a lower median eliciting dose (10 [IQR, 4-42] g) than salmon, a β-parvalbumin-poor fish (48.1 [IQR, 29-80] g; $P = .007$). The severity of AD, as defined by SCORing AD (SCORAD), was assessed in individuals with fish allergy who underwent OFCs. The median SCORAD index was 14.4 (IQR, 0-23.4). Most (59 of 73, 80%) had mild AD (SCORAD $\leq$ 25), 9 (12%) had moderate AD (SCORAD, 26-49), and 5 (9%) had severe AD (SCORAD $\geq$50). The SCORAD levels were most significantly correlated with levels of catfish-sIgE ($r = 0.470$, $P < .001$) and tilapia-sIgE ($r = 0.464$, $P < .001$), but not with tuna-sIgE ($r = 0.087$, $P = .464$) and total IgE ($r = 0.177$, $P = .169$) (Table E1, available in this article's Online Repository at www.jaci-inpractice.org).

Those who underwent OFCs had their first fish-allergic reactions at 8 (IQR, 6-10) months of age, younger than those who did not (median, 12 [IQR, 7-12] months, $P = .006$). The SPT MWDs to fish mix, catfish, and salmon were higher in those who underwent OFCs (fish mix median, 5 [IQR, 3.8-7] mm; catfish, 6.5 [4.6-8.9] mm; and salmon, 4 [2-6.1] mm) than in those who did not (fish mix median, 4 [IQR, 2-6] mm; catfish, 5 [3.5-7.1] mm; salmon, 3 [0-4.5] mm, $P < .05$) (Table I). The fish-sIgE levels were otherwise comparable in patients who had OFCs and those who did not (Figure E5, available in this article's Online Repository at www.jaci-inpractice.org). Figure 1 shows a gradient of sIgE titers against fish species with decreasing β-parvalbumin levels. The levels of fish-sIgE were higher against species with higher β-parvalbumin levels, such as tilapia, catfish, and carp, as previously reported,[22-26] than against fish with lower β-parvalbumin levels, such as tuna, salmon, and cod ($P < .001$; Figure 1 and Figure E4, available in this article's Online Repository at www.jaci-inpractice.org). In this "fish allergenicity ladder," tilapia, catfish, and GC had higher levels of allergenicity than grouper and herring, which had intermediate levels of allergenicity, and cod, halibut, salmon, and tuna, which had lower levels of allergenicity. This gradient of IgE reactivity can guide the diagnosis of individuals who are allergic to fish but show a selective tolerance to specific types of fish.

Cross-sensitization to multiple fish species was common, with 218 (88%) individuals sensitized to 3 or more fish species. Correlation was strongest between grouper and catfish among the challenged participants (Pearson's correlation coefficient $r = 0.988$, $P < .001$) and between catfish and tilapia among the nonchallenged participants ($r = 0.972$, $P < .001$). Correlation was the weakest between tuna and GC among the challenged participants ($r = 0.247$, $P < .05$) and between tuna and herring among the nonchallenged participants ($r = 0.132$, $P > .05$; Figure E3, *A* and *B*). For component testing, the IgE titers to rCyp c 1 were generally higher than those to other fish extracts. Strong correlations were observed between rGad c 1 and catfish ($r = 0.967$, $P < .001$ in the challenged participants; $r = 0.980$, $P < .001$ in the nonchallenged participants) and between rGad c 1 and tilapia ($r = 0.948$, $P < .001$ in the challenged participants; $r = 0.956$, $P < .001$ in the nonchallenged participants), most likely because of the comparably high levels of β-parvalbumin in these fish species.

Fish tolerance patterns in fish-allergic participants

One hundred (40%) fish-allergic participants, including 80 (46%) challenged and 20 (27%) nonchallenged participants, indicated tolerance to certain fish species (Table I), ranging in number from 1 to 16. Participants reported a total of 255 episodes where they were able to tolerate eating fish. The most common fish species to which the participants demonstrated tolerance were salmon (28.5%), tuna (9.2%), and halibut (8.0%), whereas tilapia (1.6%), catfish (2.4%), and carp (4.8%) were the least tolerated fish species (Table E2, available in this article's Online Repository at www.jaci-inpractice.org). A total of 1047 episodes of allergic reactions to fish were reported (data not shown). Fish species that most frequently triggered fish-allergic

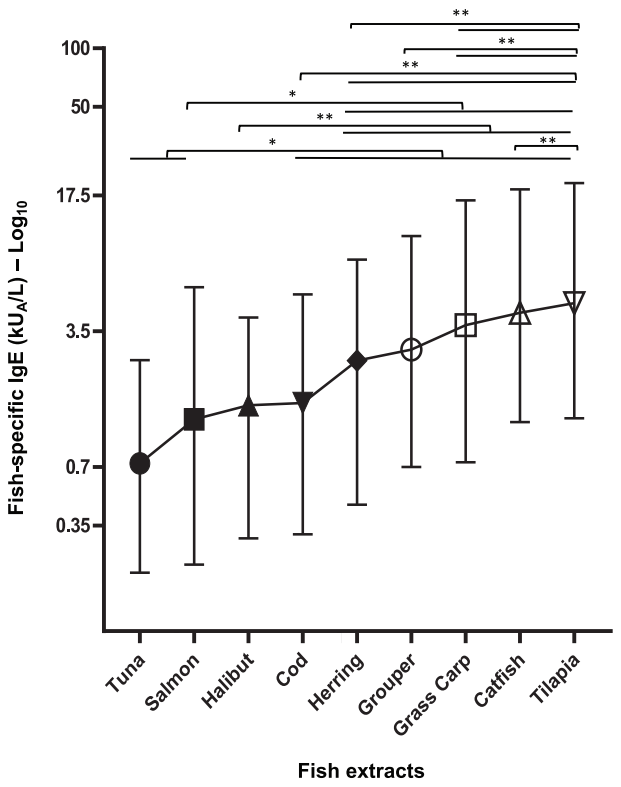

FIGURE 1. Specific IgE titers against tuna, salmon, halibut, cod, herring, grouper, grass carp, catfish, and tilapia measured using the ImmunoCAP assay. The symbol and error bar indicate the median value and interquartile range for each ImmunoCAP, respectively. **Correlation is significant at the .01 level (2-tailed). *Correlation is significant at the .05 level (2-tailed).

reactions were carp (15.1%), followed by salmon (13.6%), grouper (11.6%), and cod (7.8%) (Figure 2).

Shellfish sensitization in fish-allergic participants

Cosensitization to shellfish was noted in 130 (52%) fish-allergic participants 2.6 (IQR, 1.4-6.3) years after patients' initial fish-allergic reactions. DBPCFCs with tiger prawn were performed in 18 (7%) participants, of whom 13 (72%) failed the challenge (Table E3, available in this article's Online Repository at www.jaci-inpractice.org). Of those who failed the tiger prawn challenges, SPT to shellfish was negative in 2 individuals. All 5 individuals who had passed the tiger prawn challenges were sensitized to shellfish. The median (IQR) eliciting dose of positive tiger prawn challenges was 35.2 (20.7-91.3) g, which was equivalent to 7.0 (4.1-18.3) g of shrimp protein. Angioedema was noted in 10 (77%) individuals, rash in 7 (54%), and urticaria in 6 (46%). Two participants presented with cough and abdominal pain, whereas 1 participant developed shortness of breath without wheezing or desaturation during the challenge. Those with fish and shellfish cosensitization or allergy were recruited at 5.4 (IQR, 2.9-11.1) years of age, older than those without (median, 3.2 [IQR, 2-6.6] months, $P \leq .001$). Among those with fish and shellfish cosensitization or allergy, 85 (65%) had multiple food allergies and 65 (53%) had adrenaline autoinjector prescriptions, compared with 58 (49%) and 42 (41%) among those without ($P \leq .05$, Table E4, available in this article's Online Repository at www.jaci-inpractice.org). The proportions of participants with asthma, allergic rhinitis, and AD were otherwise similar between the 2 groups. The SPT MWDs, fish-sIgE titers, and total IgE levels were higher among those with fish and shellfish cosensitization than among those with fish sensitization only, reflecting a higher degree of atopy in the former participants. When fish-sIgE levels were corrected by total IgE levels, the fish-sIgE titers were not substantially different between the 2 groups. The level of *D.p.* sensitization was higher in those with fish and shellfish cosensitization (median, 6 [IQR, 4-9]) than in those with fish sensitization only (median, 5.5 [IQR, 2.9-7.6], $P = .038$).

Shellfish consumption patterns in fish-allergic participants

Among the fish-allergic participants without shellfish cosensitization, 39 (33%) avoided both crustaceans and mollusks, 13 (11%) avoided crustaceans, and 8 (7%) avoided mollusks (Table E5, available in this article's Online Repository at www.jaci-inpractice.org). Among individuals who reported consumption of shellfish, 61 (51%) had shrimp, 43 (36%) had crab, and 41 (35%) had clam and scallop. In contrast, in the fish and shellfish cosensitization group, 23 (18%) participants tolerated both crustaceans and mollusks, 20 (15%) tolerated mollusks, and 10 (8%) tolerated crustaceans. Shrimp was consumed by 26 (20%) of the fish- and shellfish-cosensitized participants, followed by squid/cuttlefish in 25 (19%), crab in 23 (18%), scallop in 20 (15%), and clam in 19 (15%) (Figure 3). The participants who avoided shellfish were younger, with a median age of 4.7 (IQR, 2.6-8.1) years, than those who tolerated both crustaceans and mollusks (14.7 [IQR, 10.3-15.4] years, $P = .001$; Table E6, available in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

In this study, we comprehensively evaluated the clinical characteristics, sensitization patterns, and clinical tolerance to seafood including fish, crustaceans, and mollusks. We identified a gradient of IgE sensitization to fish corresponding to their β-parvalbumin levels. More fish-allergic participants were able to tolerate β-parvalbumin-poor fish, such as tuna, salmon, and cod, than β-parvalbumin-rich fish, such as tilapia, catfish, and carp. Furthermore, nearly half of the fish-allergic participants opted to avoid shellfish after their initial fish-allergic reactions, and most were later shown to lack IgE sensitization to shellfish. In contrast, although shellfish cosensitization was observed in half of the fish-allergic participants, at least a quarter of them could tolerate crustaceans and mollusks.

Fish is a common food allergen and particularly affects young atopic children. The median age at fish allergy diagnosis in our study cohort was 4.2 years, in line with recent studies that have reported that fish allergy often presents in the first 5 years of life.[14,27,28] Mucocutaneous features, including angioedema (68%) and urticaria (33%), were the predominant fish allergy symptoms in our cohort, consistent with previous studies.[28,29] One in 6 fish-allergic participants developed an anaphylactic reaction to fish, a rate that was similar to those in other retrospective studies (10%-20%),[14,29] while AD was a common comorbidity, consistent with a prior study.[27] The age at first fish-allergic reaction in our Chinese cohort was, however, mostly

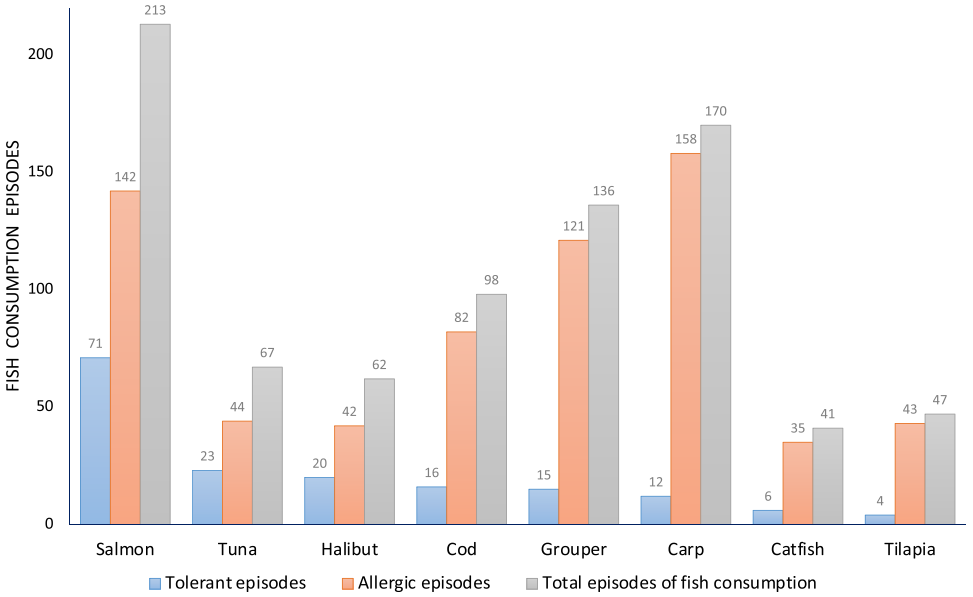

FIGURE 2. The gray, red, and blue bars represent the reported total episodes of fish consumption and allergic and tolerant episodes to various fish species, respectively.

between 7 and 12 months, as shown in Table I, which is much lower than the age at first fish-allergic reaction reported in Greek (median, 1.25 years)[27] and Australian (median, 2 years)[14] cohorts. The choice of fish was also different between Chinese and Western cultures. In Australia, 40% of children were allergic to either salmon or tuna,[14] whereas 20% of European children reacted predominantly to cod,[6] and 50% and 29% of Portuguese participants reacted to hake and mackerel, respectively.[28] Although these studies were conducted in coastal regions with an abundant supply of fish, the difference in fish allergy presentation was likely attributable to the cultural differences across regions. Chinese cuisine often favors freshwater fish (ie, β-parvalbumin-rich fish) because of their lower mercury levels,[17,27] abundant supply, and reduced cost. GC, the top trigger of fish-allergic reactions in our cohort, is the typical freshwater fish used to make rice porridge (congee) because of its rich nutrition and few bony spurs. GC congee, which combines deboned and mashed fish flesh with congee cooked with fish bone and rice, yields a smooth-textured meal suitable for infants aged 4 to 6 months and above. In contrast, fish that has been fried or baked in Western cooking is typically cut up into chunks of flesh that usually older infants with teeth appreciate. In an Infant Feeding Survey conducted in the United Kingdom, fish was consumed less than once a week or never by 44% of 8- to 10-month-old infants.[30] Here, we observe that early exposure to β-parvalbumin-rich fish is associated with an early onset of fish allergy. In contrast, exposing at-risk children to fish with lower levels of β-parvalbumin may delay the onset of fish allergy. This hypothesis requires further validation by experimental research. Yet, the clinical information provided by this study prompts a timely diagnosis of fish allergies, particularly in regions where the consumption of β-parvalbumin-rich fish is high.

The differential parvalbumin levels in fish species are related to their habitats. Marine groundfish (also known as demersal fish), such as cod and grouper, have a higher proportion of white muscle, which is composed of fast-twitch fibers and used for both prey capture and escape maneuvers, than dark muscle. In contrast, swarm fish (or pelagic fish), such as tuna and salmon, have a higher proportion of dark muscle that allows for continuous swimming. Freshwater fish, including GC, catfish, and tilapia, examined in this study have also been reported to contain a high proportion of white muscle and a high level of parvalbumin.[23,31] In this study, the amount of β-parvalbumin in fish corresponded to the participants' fish-sIgE titers. Although our patients demonstrated sensitization to multiple fish species across different fish families, their IgE titers to large-sized pelagic fish, such as tuna and salmon—fish species that typically have higher dark muscle contents—were significantly lower, as shown in Figure 1 and Figure E4, available in this article's Online Repository at www.jaci-inpractice.org. In contrast, their IgE titers to demersal and freshwater fish, such as grouper, catfish, and carp, which are typically bottom feeders with higher white muscle contents, were higher. Studies have shown that fish dark muscle is less allergenic than white muscle because of the lower parvalbumin levels in the former.[22,23,31] This also corroborates a finding of our previous study that demonstrated a higher IgE reactivity to GC parvalbumin (Cten i 1) than to cod and salmon.[24] Cross-inhibition enzyme-linked immunosorbent assay further showed that the parvalbumin levels in cod (Gad m 1) and salmon (Sal s 1) were only able to inhibit 60% to 70% of IgE binding against Cten i 1. In this study, IgE titers were highest against tilapia, catfish, and GC (ie, fish species with higher levels of allergenicity), followed by grouper and herring (which have intermediate levels of allergenicity), and were lowest against cod, halibut, salmon, and tuna (which have lower levels of allergenicity). Alternative fish extracts, such as catfish, can be used in areas where GC is less often consumed or available, much as herring can be used in place of grouper. This concept has been corroborated by our clinical data showing that the top fish species with reported tolerance in our cohort were salmon, tuna, and

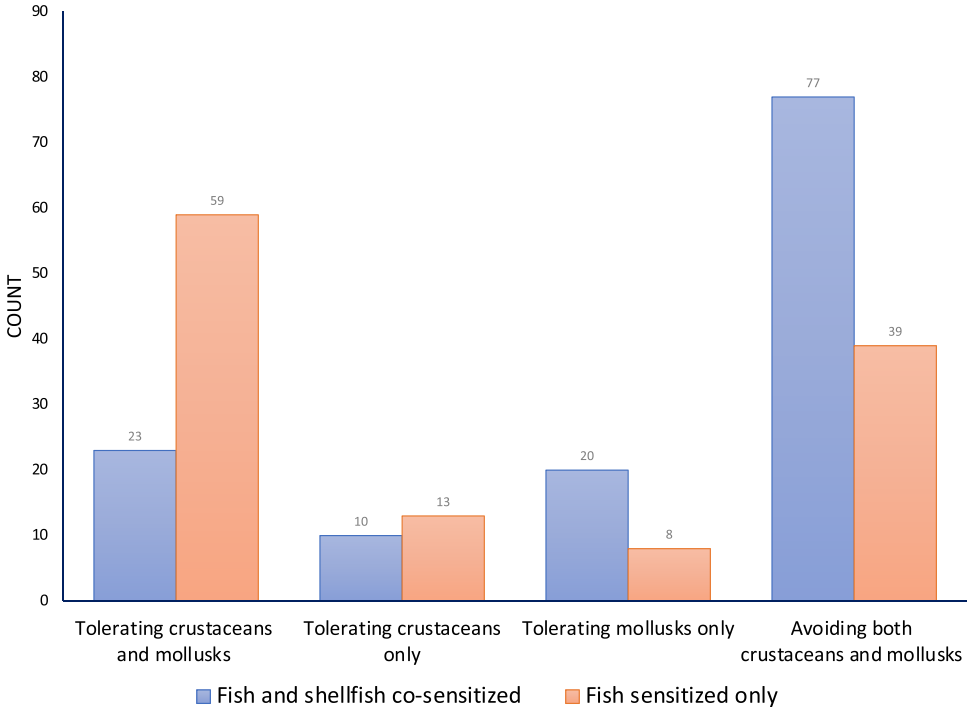

FIGURE 3. Comparison of the consumption pattern of crustaceans and mollusks in individuals with fish and shellfish cosensitization and those with fish sensitization only.

halibut, whereas white fish species such as catfish and carp had the lowest tolerance levels. These tolerance patterns, again, corresponded to the β-parvalbumin contents and serological IgE reactivities described earlier. Furthermore, our results indicated that the tolerance pattern to fish was not directly reflective of societal eating habits. Salmon was the most consumed fish with the highest tolerance level among our fish-allergic subjects. In contrast, carp was the second most consumed fish among our population, but it was one of the least tolerated fish (Figure 2). In our study, 40% of the fish-allergic participants reported tolerance to at least 1 fish species, a rate that was similar to our DBPCFC study in which 48% of our challenged participants failed either GC or salmon OFCs (Table I). This rate is higher than that reported in the study by Sørensen et al,[6] in which 29% of the fish-sensitized participants were selectively tolerant to at least 1 fish species.[32] Clinical tolerance to salmon and halibut has been less commonly reported in the literature, whereas tuna and swordfish are the fish species to which Greek children with persistent fish allergy are most tolerant.[27] Fish has been shown to be less allergenic when canned,[33] as demonstrated in a Spanish cohort in which all of the 24 fish-allergic individuals tolerated 90 g of canned tuna in OFCs.[34] Collectively, findings from these studies suggest that selective tolerance to fish is not uncommon. Individuals with lower levels of sIgE titers against β-parvalbumin-poor fish may have selective tolerance to these species, even if they have a fish allergy. Choosing fish species with lower levels of β-parvalbumin is safer for OFCs.

Shellfish cosensitization was noted in nearly half of our fish-allergic participants, although data on true shellfish co-allergy informed by OFCs were only available in 7% of our cohort. A previous survey in the United States found that the lifetime prevalence of reported seafood allergy in the general population was 2.3%, with approximately 10% of them reporting allergies to both shellfish and fish.[18] In Australia, 49% of crustaceans-allergic children were found to be sensitized to fish, given that a majority of them had experienced prior clinical allergic reactions to fish.[14] In this study, shellfish cosensitization occurred more commonly in those who were older, had multiple food allergies, and owned an adrenaline device, although their comorbid conditions, including AD and asthma, and fish allergy symptom profiles were similar (Table E4, available in this article's Online Repository at www.jaci-inpractice.org). Initial analysis revealed that the participants with fish and shellfish cosensitization had higher fish and total sIgE titers than those with fish sensitization only. After adjusting the fish-sIgE with the total IgE levels, the fish-sIgE to total IgE levels were similar between these 2 groups; thus, fish-sIgE may not be a useful marker to predict shellfish co-allergy. Sensitization to shellfish was similarly shown to be a poor predictor of clinical shellfish allergy. In our study, all 5 subjects who were challenged negative to shrimp were sensitized to shellfish, and of these shellfish-cosensitized individuals, 40% reported ever tolerating crustaceans or mollusks, as shown in Tables E3 and E5 in this article's Online Repository at www.jaci-inpractice.org. It will be of interest to investigate whether sIgE to novel shrimp allergens recently reported by our group may help predict fish and shellfish co-allergy.[35] Among our participants who were enrolled 2.2 ± 3.4 years after their initial fish-allergic reactions, 116 (47%) reported avoidance of all shellfish species, of whom only 66% were subsequently found to have shellfish cosensitization (Table E6, available in this article's Online Repository at

www.jaci-inpractice.org). Patients with a fish allergy do not need to avoid shellfish unless they have had an adverse reaction to it. However, it is crucial to evaluate patients with cosensitization to shellfish, especially in regions like China where house dust mites are prevalent, through OFCs. Current diagnostic assays have limited accuracy in shellfish diagnosis due to cross-reactivity with dust mites.

The limitation of this study was that only 75 (30%) subjects were confirmed to be allergic to fish by OFCs. Although the rest of the cohort was diagnosed based on their clinical history and fish sensitization, most demographic and clinical characteristics and fish-sIgE levels were comparable between the 2 groups. Although we measured the patients' sIgE levels to an array of fish species, only OFCs with GC, grouper, salmon, and cod were arranged for our participants. It remains a drawback that some participants with low fish-sIgE levels were not subjected to OFCs with corresponding species. In this study, we only quantified sIgE levels against parvalbumin, excluding other minor allergens, such as collagen, enolase, aldolase, and tropomyosin. In our previous study, however, we reported that sensitization to heat-labile allergens, such as enolase and aldolase, was low in our Chinese fish-allergic cohort.[24] IgE sensitization to mollusks other than clam and scallop was not examined, but this did not significantly affect our evaluation of the shellfish avoidance pattern. The clinical information collected on previous fish-allergic reactions and seafood consumption patterns may be subject to recall bias. As this study involved multiple centers, we were unable to document doctors' advice for each of the participants accurately. Lastly, the proposed gradient of IgE sensitization can be used as a "fish allergenicity ladder" tailored for Asian populations, but for other ethnic groups, it is necessary to consider geographical and cultural factors when developing a locally applicable "ladder" for use in clinical settings.

In conclusion, fish allergy can develop during childhood in the first few years of life and potentially cause anaphylaxis. Despite the notion that cross-sensitization to multiple fish species is common, 40% of our participants reported clinical tolerance to 1 or more fish species. We observed a relationship between the level of parvalbumin in fish and the gradient of IgE sensitization. Our clinical data on fish tolerance patterns further support this finding. We propose a ladder system for allergists to determine which fish species a patient may have selective tolerance to despite suffering from a fish allergy. This system, called the "fish allergenicity ladder," is designed to help allergists make informed choices and minimize the risk of adverse reactions during OFCs. Choosing fish with lower levels of β-parvalbumin is recommended as a safer option. We also highlighted the complexity of shellfish allergy, of which at least a quarter of our participants did not exhibit clinical allergy to shellfish despite showing IgE sensitization. Individuals allergic to fish, irrespective of shellfish sensitization, tend to avoid shellfish. Timely dietary counseling and performing OFCs for suspected shellfish-allergic individuals to confirm or exclude the diagnosis of crustaceans and mollusk allergies is crucial, as with examining the rate of unnecessary shellfish avoidance in individuals with other food allergies.

Further prospective challenge-based studies are underway to evaluate patients' clinical and immunological reactivity to different fish and shellfish species, develop diagnostic tests with improved accuracy, and promote precision medicine for seafood allergy. Further study should be performed to assess the risk of allergic reactions caused by fish cross-contamination, especially when dining out. Until more information is available, it is recommended that individuals with selective fish tolerance consume tolerant fish at home in a careful and gradual manner, following a thorough evaluation and counseling.

Acknowledgments

The authors thank Jaimie Ngai, Yuki Shum, Cherry Yung, Maco Lam, Rebecca Li, Tiffany Kwok, Kary Xu, Kevin Cheng, and Tik Wai Fu for performing skin prick tests, specimen processing, subject interview, and data management. They also thank our nurses Tiffany Tavares, Suk Tak Lee, Po Yin Cheng, Annerliza Kwok, Cecily Leung, Vivian Yu, and Ka Sheung Yip for performing OFCs for our patients. The authors would like to thank all the children and their families for their participation in this study.

A. S. Y. Leung and T. F. Leung was responsible for concept and design of this study. A. S. Y. Leung and C. Y. Y. Wai were responsible for the acquisition of data and A. S. Y. Leung was responsible for the analysis of data. A. S. Y. Leung, T. F. Leung and C. Y. Y. Wai obtained funding for the allergy tests and OFCs. All authors were responsible for drafting and revision of the final paper.

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ONLINE REPOSITORY

Participants and allergy assessment

The study population included patients with a clinical history of fish allergy (n = 249) recruited from 6 hospitals in Hong Kong, namely Prince of Wales Hospital (PWH), Queen Elizabeth Hospital (QEH), Queen Mary Hospital (QMH), Princess Margaret Hospital (PMH), Yan Chai Hospital (YCH), and United Christian Hospital (UCH), from July 2016 to December 2021. This study was reviewed and approved by the institutional review boards of respective hospitals: PWH—Joint CUHK-NTEC CREC (2017.542); QEH—KC/KE REC (KC/KE-17-0217/FR-4); QMH—HKU/ HA HKW IRB (UW16-2003); PMH and YCH—KWC REC (KW/EX-18-116[127-12]); UCH—KC/KE REC (KC/KE-20-0355/ER-1).

Allergy assessment

Skin prick test with grass carp. Fresh grass carp (*Ctenopharyngodon idella*) was purchased from local fresh market and washed in distilled water. Raw and cooked (steamed at 100°C for 10 minutes) fish meat was homogenized in phosphate buffered saline in 1:10 weight to volume ratio and then filter-sterilized using 0.2 μm polyethersulfone membrane filters.

Data collection

Specific IgE level. Participants' total IgE and sIgE levels to cod (f3), tuna (f40), salmon (f41), halibut (f303), catfish (f369), grass carp (research use), grouper (f410), shrimp (f24), clam (f207), scallop (f338) and components rGad c 1 (f426), rCyp c 1 (f355), and rPen a 1 (f351) were quantified by the ImmunoCAP assay (Thermo Fisher, Uppsala, Sweden) with the Phadia 250 analyzer according to the manufacturer's instructions. The grass carp ImmunoCAP was developed as an experimental assay through our collaboration with Thermo Fisher, and not available commercially. Results were considered positive if values were greater than 0.35 kUA/L.

Oral food challenges. In selected participants, DBCPFCs with grass carp (*Ctenopharyngeon idella*), salmon (*Salmo salar*), and placebo on 3 separate days in randomized order, each at least 48 hours apart, as previously described,[E1] were arranged for fish-allergic patients with uncertain diagnoses despite clinical history, SPT, and sIgE results. Similarly, DBCPFCs with cod (*Gadus morhua*) and placebo were performed on 2 separate days using the exact dosing but different vehicles (meat for GC and salmon; potato for cod). A subgroup of patients with uncertain diagnoses underwent open-labeled challenges with grouper using the same dosing as the above. Similar DBPCFCs with tiger prawn (*Penaeus monodon*) and placebo on 2 separate days were arranged for a subgroup of patients with suspected shrimp allergy.[E2]

Allergy history data collection. Data including patient demographics, atopic comorbidities, food allergic history, and SPT and sIgE results at the time of fish allergy diagnosis were reviewed. The diagnoses of food allergy and allergic comorbidities were made by the attending physician. Participants with anaphylaxis who met the defining criteria set out by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network criteria (NIAID/FAAN)[E3] were identified. Participants and/or parents were further interviewed by the research team using a standardized questionnaire for the consumption pattern of fish (saltwater fish like cod, tuna, salmon, grouper, and bream; and freshwater fish like carp and catfish), crustacean (shrimp, crab, and lobster) and mollusk (clam, scallop, oyster, squid/cuttlefish, abalone, and mussel) in recent 2 years. Participants were categorized as having "partial tolerance" to fish if they reported tolerance and regular consumption of 1 or more fish species, while "complete avoidance" referred to patients who reported neither clinical tolerance nor regular consumption of fish (Figure E1).

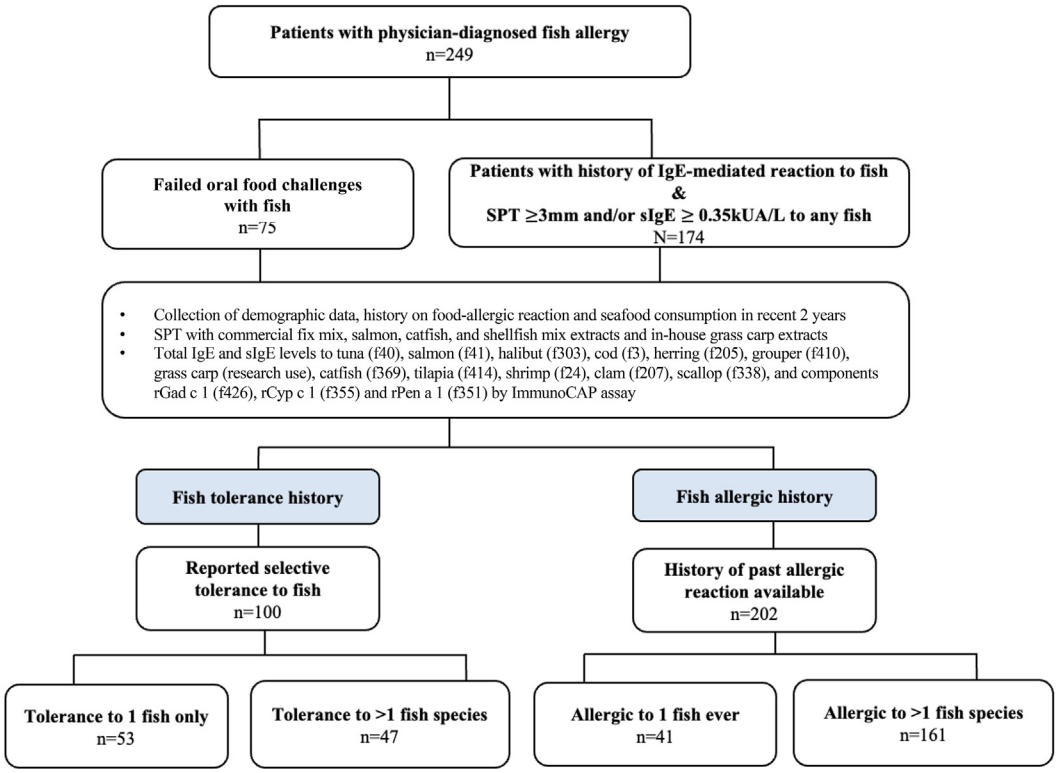


FIGURE E1. Flow diagram showing the recruitment criteria and procedures. Fish-allergic participants were further categorized based on their tolerance pattern to fish. *sIgE*, Specific IgE; *SPT*, skin prick test.

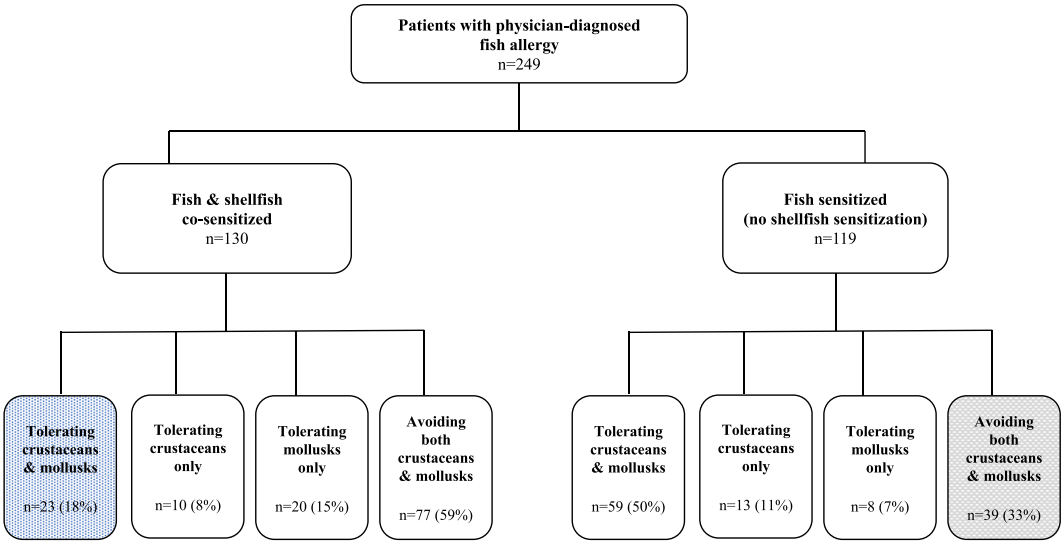


FIGURE E2. Categorization of fish-allergic participants based on their IgE sensitization statuses to crustaceans and mollusks. Participants were further divided into 4 groups based on their shellfish consumption pattern.

Correlations

	Tuna	Salmon	Halibut	Cod	Herring	Grouper	GC	Catfish	Tilapia	rGadc1	rCypc1
Tuna	1	0.687**	0.462**	0.408**	0.288*	0.401**	0.247*	0.303*	0.305**	0.308**	0.216
Salmon	0.687**	1	0.756**	0.760**	0.523**	0.746**	0.572**	0.731**	0.641**	0.660**	0.567**
Halibut	0.462**	0.756**	1	0.918**	0.834**	0.863**	0.653**	0.902**	0.897**	0.932**	0.706**
Cod	0.408**	0.760**	0.918**	1	0.759**	0.854**	0.824**	0.921**	0.815**	0.893**	0.89**
Herring	0.288*	0.523**	0.834**	0.759**	1	0.567**	0.949**	0.901**	0.886**	0.828**	0.847**
Grouper	0.401**	0.746**	0.863**	0.854**	0.567**	1	0.781**	0.988**	0.973**	0.925**	0.770**
GC	0.247*	0.572**	0.653**	0.824**	0.949**	0.781**	1	0.872**	0.800**	0.738**	0.861**
Catfish	0.303*	0.731**	0.902**	0.921**	0.901**	0.988**	0.872**	1	0.985**	0.967**	0.825**
Tilapia	0.305**	0.641**	0.897**	0.815**	0.886**	0.973**	0.800**	0.985**	1	0.948**	0.721**
rGadc1	0.308**	0.660**	0.932**	0.893**	0.828**	0.925**	0.738**	0.967**	0.948**	1	0.779**
rCypc1	0.216	0.567**	0.706**	0.890**	0.847**	0.770**	0.861**	0.825**	0.721**	0.779**	1

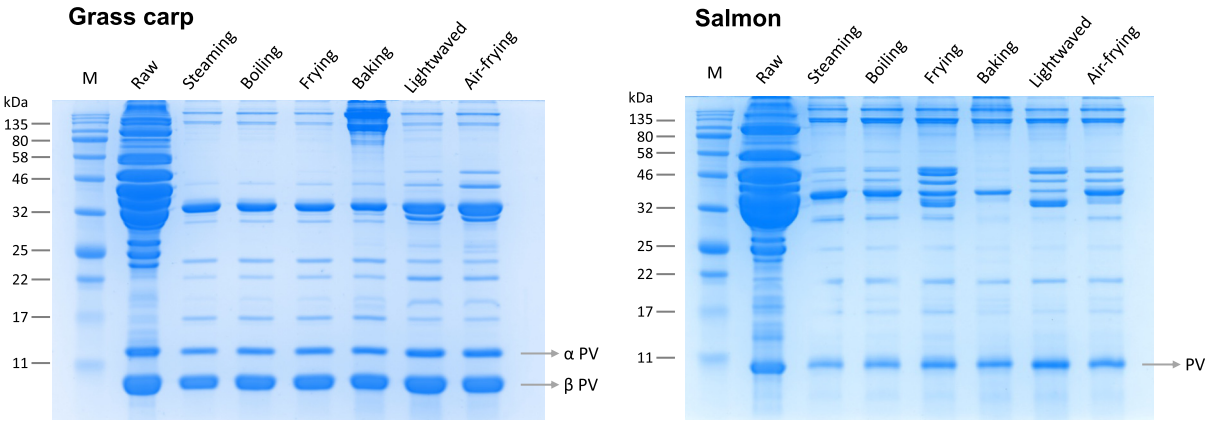
A

Correlations

	Tuna	Salmon	Halibut	Cod	Herring	Grouper	GC	Catfish	Tilapia	rGadc1	rCypc1
Tuna	1	0.776**	0.524**	0.362**	0.132	0.297**	0.268**	0.191*	0.170**	0.187**	0.181**
Salmon	0.776**	1	0.787**	0.730**	0.340**	0.640**	0.578**	0.554**	0.515**	0.528**	0.510**
Halibut	0.524**	0.787**	1	0.853**	0.599**	0.854**	0.695**	0.784**	0.735**	0.734**	0.703**
Cod	0.362**	0.730**	0.853**	1	0.523**	0.863**	0.848**	0.807**	0.754**	0.790**	0.832**
Herring	0.132	0.340**	0.599**	0.523**	1	0.466**	0.885**	0.688**	0.652**	0.672**	0.799**
Grouper	0.297**	0.640**	0.854**	0.863**	0.466**	1	0.775**	0.785**	0.727**	0.655**	0.661**
GC	0.268**	0.578**	0.695**	0.848**	0.885**	0.775**	1	0.689**	0.663**	0.612**	0.738**
Catfish	0.191*	0.554**	0.784**	0.807**	0.688**	0.785**	0.689**	1	0.972**	0.980**	0.886**
Tilapia	0.170**	0.515**	0.735**	0.754**	0.652**	0.727**	0.663**	0.972**	1	0.956**	0.868**
rGadc1	0.187**	0.528**	0.734**	0.790**	0.672**	0.655**	0.612**	0.980**	0.956**	1	0.892**
rCypc1	0.181**	0.510**	0.703**	0.832**	0.799**	0.661**	0.738**	0.886**	0.868**	0.892**	1

B

FIGURE E3. (**A**) Correlation of specific IgE (sIgE) sensitization between various fish extracts (tuna, salmon, halibut, cod, herring, grouper, grass carp, catfish, and tilapia) and fish components (rGad c 1 and rCyp c 1) among 75 fish-challenged participants. (**B**) Correlation of sIgE sensitization between various fish extracts (tuna, salmon, halibut, cod, herring, grouper, grass carp, catfish, and tilapia) and fish components (rGad c 1 and rCyp c 1) among 174 non–fish-challenged participants. **Correlation is significant at the .01 level (2-tailed). *Correlation is significant at the .05 level (2-tailed). *GC*, Grass carp.



A

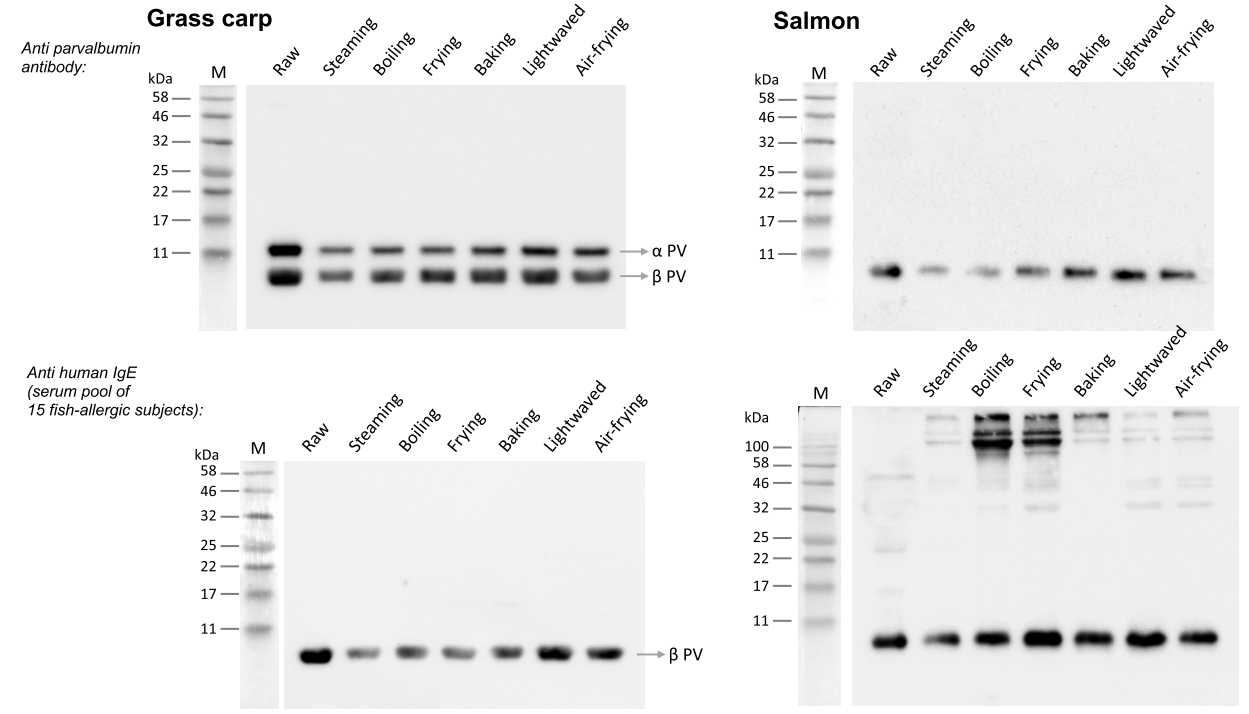
α PV & β PV % in total protein

Band %	Raw	Steaming	Boiling	Frying	Baking	Lightwaved	Air-frying
α PV	7.19	12.99	13.59	14.44	9.86	14.13	12.45
β PV	15.78	33.69	34.67	35.57	24.44	29.96	24.42
α+ β PV	**22.97**	**46.68**	**48.27**	**50.01**	**34.30**	**44.09**	**36.87**

PV % in total protein

Band %	Raw	Steaming	Boiling	Frying	Baking	Lightwaved	Air-frying
PV	7.86	16.54	17.18	18.12	30.13	27.01	19.99

P = .018, PV levels between grass carp and salmon by Wilcoxon Signed Rank test



B

FIGURE E4. Identification of IgE-binding fish proteins. (**A**) SDS-PAGE of the extracted raw grass carp and salmon proteins prepared by different cooking methods. Raw protein was diluted by 5-fold for better band visualization. Densitometry analyses were made by ImageLab. (**B**) IgE-binding proteins of grass carp and salmon extracts probed using anti-parvalbumin antibodies and sera from 15 fish-allergic subjects.

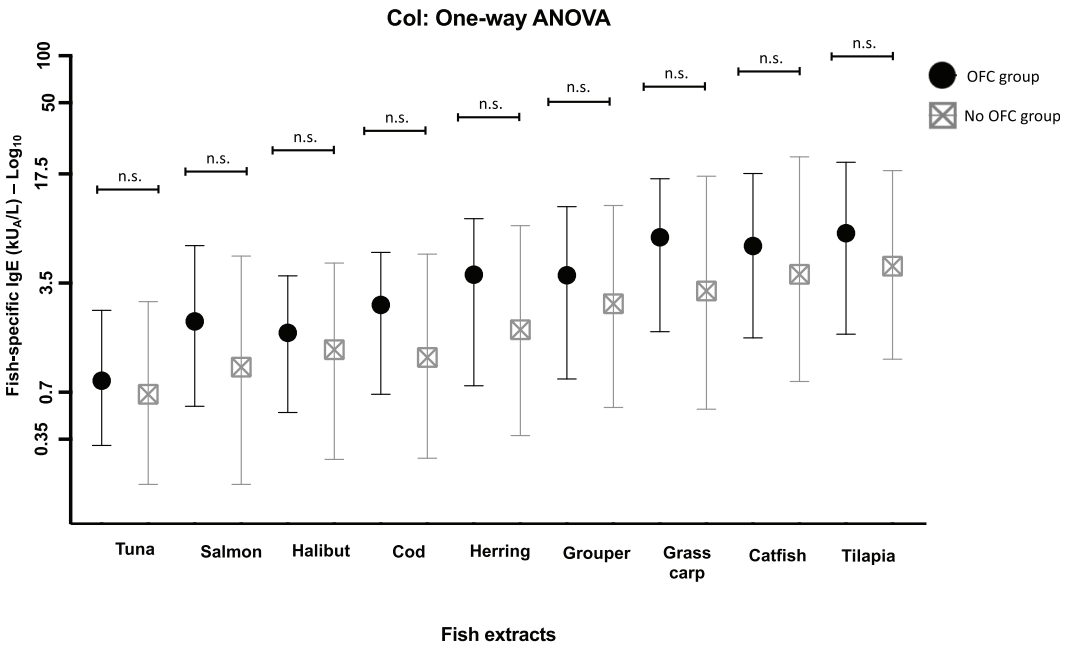


FIGURE E5. Comparison of specific IgE titers against tuna, salmon, halibut, cod, herring, grouper, grass carp, catfish, and tilapia between participants with and without oral food challenge (OFC) to fish. Levels of fish-specific IgE sensitization were comparable for all pairwise comparisons ($P > .05$). *n.s.*, Not significant.

TABLE E1. Correlation between fish-specific IgE sensitization levels and SCORAD indices among 75 participants who received challenges with fish

		95% Confidence intervals		
	Pearson correlation	Lower	Upper	*P* value
SCORAD - Tuna	0.087	−0.146	0.311	.464
SCORAD - Salmon	0.389	0.175	0.568	**<.001**
SCORAD - Halibut	0.386	0.163	0.572	**.001**
SCORAD - Cod	0.350	0.131	0.537	**.002**
SCORAD - Herring	0.245	0.004	0.460	**.047**
SCORAD - Grouper	0.361	0.143	0.546	**.002**
SCORAD - GC	0.292	0.063	0.492	**.014**
SCORAD - Catfish	0.470	0.257	0.639	**<.001**
SCORAD - Tilapia	0.464	0.261	0.627	**<.001**
SCORAD - rGadc1	0.453	0.249	0.619	**<.001**
SCORAD - rCypc1	0.338	0.116	0.529	**.004**

GC, Grass carp; *SCORAD*, SCORing Atopic Dermatitis.
Significant values ($P < .05$) are bolded.

TABLE E2. Fish species that were reported to be tolerant in our fish-allergic population, and their corresponding genus, family, and order

Fish common names	Genus	Family	Order	n (%)
Salmon	*Salmo*	*Salmonidae*	Salmonidae	71 (27.84)
Tuna	*Thunnus*	*Scombridae*	Scombriformes	23 (9.02)
Halibut/flatfish	*Hippoglossus*	*Pleuronectidae*	Pleuronectiformes	20 (7.84)
Cod	*Gadus*	*Gadidae*	Gadiformes	16 (6.27)
Golden threadfin bream	*Nemipterus/Acanthopagrus*	*Nemipteridae*	Perciformes	16 (6.27)
Grouper	*Plectropomus/Epinephelus*	*Serranidae*	Perciformes	15 (5.88)
Bigeye fish	*Priacanthus*	*Priacanthidae*	Perciformes	13 (5.10)
Carp	*Ctenopharyngodon/Cirrhinus*	*Cyprinidae*	Cypriniformes	12 (4.71)
Eel	*Congresox*	*Muraenesocidae*	Anguilliformes	12 (4.71)
Yellow croaker	*Larimichthys*	*Sciaenidae*	Acanthuriformes	10 (3.92)
Pomfret	*Pampus*	*Stromateidae*	Perciformes	9 (3.53)
Mandarin fish	*Siniperca*	*Sinipercidae*	Perciformes	8 (3.14)
Catfish	*Silurus*	*Siluridae*	Siluriformes	6 (2.35)
Mullet	*Mugil*	*Mugilidae*	Mugiliformes	6 (2.35)
Herring	*Clupea*	*Clupeidae*	Clupeiformes	4 (1.57)
Tilapia	*Coptodon*	*Cichlidae*	Cichliformes	4 (1.57)
Others				10 (3.92)

TABLE E3. Comparison of the clinical and serological profiles between individuals who passed oral food challenges (OFCs) with tiger prawn and those who failed OFCs with tiger prawn

Characteristics	Total subjects (N = 18)	Failed OFC (n = 13)	Passed OFC (n = 5)	*P* value*
Age (y), median (IQR)	7.46 (3.87-13.50)	7.71 (3.29-14.04)	7.20 (4.95-22.30)	.849
Male, n (%)	13 (72.22)	9 (69.23)	4 (80.00)	.648
sIgE to shrimp (kUA/L), median (IQR)	10.04 (6.06-20.30)	8.97 (8.27-15.80)	11.10 (8.21-20.40)	.864
sIgE to rPen a 1 (kUA/L), median (IQR)	6.00 (0.50-10.07)	5.75 (0.50-8.46)	7.04 (3.55-14.52)	.864
SPT to shellfish (mm), median (IQR)	4.25 (3.00-7.00)	5.00 (2.00-8.50)	4 (3.50-4.50)	.924

IQR, Interquartile range; *sIgE*, specific IgE; *SPT*, skin prick test.
**P* value between individuals who passed OFCs to tiger prawn and those who failed.

TABLE E4. Comparison of clinical and serological characteristics between participants with fish and shellfish cosensitization and those with fish sensitization only (N = 249)

Characteristics	Fish and shellfish cosensitized (n = 130)	Fish sensitized only (n = 119)	*P* value
Age (y), median (IQR)	5.36 (2.90-11.14)	3.20 (1.98-6.62)	**<.001**
Male, n (%)	92 (70.77)	72 (60.50)	.088
Asthma, n (%)	30 (23.43)	39 (33.05)	.094
Allergic rhinitis, n (%)	78 (60.93)	59 (50.00)	.084
Atopic dermatitis, n (%)	121 (94.53)	113 (95.76)	.654
Other food allergies, n (%)	85 (65.38)	58 (49.15)	**.010**
Own an AAInj, n (%)	65 (53.72)	42 (40.78)	**.029**
Age from fish-allergic reaction to recruitment (y), median (IQR)	2.55 (1.42-6.33)	1.81 (0.78-3.87)	.059
Sensitization profile to fish, median (IQR)			
SPT result (mm)			
SPT to fish mix	5.00 (3.50-7.00)	3.50 (2.00-5.50)	**.001**
SPT to salmon	4.00 (3.00-5.00)	3.00 (0.00-4.00)	**<.001**
SPT to catfish	6.00 (4.00-8.00)	5.00 (3.50-7.50)	.183
Serological IgE result (kUA/L)			
Specific IgE to cod	1.76 (0.72-4.63)	1.18 (0.27-4.81)	**<.001**
Specific IgE to tuna	0.67 (0.34-2.10)	0.58 (0.14-1.38)	**<.001**
Specific IgE to salmon	1.38 (0.62-5.19)	1.73 (0.16-5.24)	**<.001**
Specific IgE to halibut	1.52 (0.51-2.66)	0.86 (0.17-2.95)	**.001**
Specific IgE to herring	3.26 (0.79-11.05)	1.47 (0.33-5.90)	**.005**
Specific IgE to catfish	4.45 (1.27-9.55)	3.32 (1.05-14.90)	**.006**
Specific IgE to grouper	3.93 (1.24-18.50)	2.09 (0.41-6.86)	**.002**
Specific IgE to grass carp	4.80 (1.60-11.50)	2.86 (0.84-14.25)	**.001**
Specific IgE to tilapia	7.12 (1.53-33.55)	3.27 (0.87-13.70)	**.001**
Specific IgE to rGad c 1	2.79 (0.88-6.49)	2.86 (0.68-10.30)	**.001**
Specific IgE to rCyp c 1	4.89 (1.56-9.25)	4.37 (1.69-15.80)	**<.001**
Total IgE (kUA/L)	762 (349-2350)	566 (193-871)	**.003**
Specific IgE: total IgE ($\times 10^3$)			
Cod-sIgE: total IgE	3.02 (0.51-8.48)	2.86 (0.45-10.09)	.871
Tuna-sIgE: total IgE	1.12 (0.4-3.62)	1.19 (0.26-3.06)	.626
Salmon-sIgE: total IgE	2.44 (0.7-8.2)	2.38 (0.29-9.04)	.576
Halibut-sIgE: total IgE	1.98 (0.52-4.78)	1.91 (0.29-4.61)	.730
Herring-sIgE: total IgE	3.61 (0.69-11.9)	5.01 (0.56-16.06)	.933
Catfish-sIgE: total IgE	6.94 (1.4-26.05)	8.59 (1.21-27.65)	.781
Grouper-sIgE: total IgE	3.97 (0.96-12.37)	4.49 (0.75-14.44)	.749
Grass carp-sIgE: total IgE	8.37 (1.73-24.6)	8.4 (2.19-33.86)	.599
Tilapia-sIgE: total IgE	8.47 (1.73-29.63)	11.25 (1.66-37.72)	.667
rGad c 1-sIgE: total IgE	6.16 (1.16-20.09)	7.81 (1.06-21.17)	.978
rCyp c 1-sIgE: total IgE	7.92 (1.84-32.84)	12.13 (1.58-36.21)	.608
Failed fish OFC, n (%)	44 (33.85)	31 (26.05)	.180
Sensitization profile to shellfish, median (IQR)			
SPT result (mm)			
SPT to shellfish	4.50 (3.00-6.00)	0.00 (0.00-0.00)	**<.001**
Serological IgE result (kUA/L)			
Specific IgE to shrimp	8.97 (3.54-22.3)	0.14 (0.04-0.23)	**<.001**
Specific IgE to rPen a 1	5.75 (0.49-18.5)	0.03 (0.01-0.06)	**<.001**
Specific IgE to crab	6.83 (0.82-19.8)	0.10 (0.03-0.15)	**<.001**
Specific IgE to clam	1.25 (0.34-3.21)	0.04 (0.03-0.09)	**<.001**
Specific IgE to scallop	2.00 (0.72-8.13)	0.08 (0.04-0.13)	**<.001**
SPT result to *D.p.* (mm)	6.00 (4.00-9.00)	5.50 (2.88-7.63)	**.038**
SPT result to *D.f.* (mm)	5.75 (3.00-9.00)	4.50 (3.00-7.75)	.117

AAInj, Adrenaline autoinjector; *D.f.*, *Dermatophagoides farinae*; *D.p.*, *Dermatophagoides pteronyssinus*; *IQR*, interquartile range; *SPT*, skin prick test.
Significant values ($P < .05$) are bolded.

TABLE E5. Comparison of shellfish consumption pattern between participants with fish and shellfish cosensitization and those with fish sensitization only

Characteristics	Total (N = 249)	Fish and shellfish cosensitized (n = 130)	Fish sensitized only (n = 119)	*P* value*
Shrimp OFC result, n (%)				
Failed shrimp OFC	13 (5.2)	11 (8.5)	2 (1.7)	.352
Shellfish consumption pattern, n (%)				**<.001**
Avoiding both crustaceans and mollusks	116 (46.6)	77 (59.2)	39 (32.8)	
Tolerating crustaceans and mollusks	82 (32.9)	23 (17.7)	59 (49.6)	
Tolerating crustaceans only	23 (9.2)	10 (7.7)	13 (10.9)	
Tolerating mollusks only	28 (11.2)	20 (15.4)	8 (6.7)	
Crustacean consumption, n (%)				
Shrimp	87 (34.9)	26 (20.0)	61 (51.3)	**<.001**
Crab	66 (26.5)	23 (17.7)	43 (36.1)	**<.001**
Lobster	37 (14.9)	11 (8.5)	26 (21.8)	**.003**
Not specified	8 (3.2)	1 (0.8)	7 (5.9)	**.022**
Mollusks consumption, n (%)				
Clam	60 (24.2)	19 (14.7)	41 (34.5)	**<.001**
Scallop	61 (24.5)	20 (15.4)	41 (34.5)	**<.001**
Oyster	22 (8.8)	7 (5.4)	15 (12.6)	**.045**
Mussel	33 (13.3)	9 (6.9)	24 (20.2)	**.002**
Squid/cuttlefish	64 (25.7)	25 (19.2)	39 (32.8)	**.015**
Abalone	47 (18.9)	15 (11.5)	32 (26.9)	**.002**
Not specified	10 (4.0)	3 (2.3)	7 (5.9)	.151

OFC, Oral food challenge.
Significant values ($P < .05$) are bolded.
**P* value between individuals with fish and shellfish cosensitization and those with fish sensitization only.

TABLE E6. Comparison of the clinical and serological characteristics of participants with different shellfish consumption patterns

Shellfish consumption pattern	Avoiding both crustaceans and mollusks	Tolerating crustaceans and mollusks	Tolerating crustaceans only	Tolerating mollusks only	*P* value
Fish-allergic subjects, n (%)	n = 116	n = 82	n = 23	n = 28	
Subjects with shellfish sensitization	77 (66.38)	23 (28)	10 (43.5)	20 (71.4)	**<.001**
Subjects with positive OFC to shrimp	6 (5.17)	1 (1.2)*	1 (4.3)*	5 (17.9)	.597
Age (y), median (IQR)	4.68 (2.64-8.04)	14.7 (10.32-15.37)	9.77 (5.72-13.9)	6.58 (6.0-10.56)	**.001**
Skin prick testing (mm), median (IQR)					
SPT to fish mix	5.0 (3.00-7.00)	4.00 (2.00-6.00)	5.00 (3.00-7.00)	3.50 (0.50-6.00)	.064
SPT to salmon	3.50 (2.00-5.00)	3.00 (0.50-5.00)	3.50 (0.00-5.25)	2.25 (0.00-3.88)	.108
SPT to shellfish	3.50 (0.00-6.00)	0.00 (0.00-2.63)	0.00 (0.00-3.63)	3.50 (2.50-5.88)	**<.0001**
Serological IgE (kUA/L), median (IQR)					
Specific IgE to shrimp	8.65 (2.77-21.85)	1.41 (0.26-4.94)	0.10 (0.08-4.70)	11.50 (2.34-62.40)	**.013**
Specific IgE to rPen a 1	7.04 (0.60-18.30)	0.04 (0.03-1.18)	0.04 (0.01-4.67)	1.24 (0.69-44.00)	**.001**
Specific IgE to crab	6.88 (0.41-20.30)	0.24 (0.13-1.35)	0.10 (0.03-7.33)	5.74 (0.82-42.30)	**.012**
Specific IgE to clam	1.75 (0.32-3.89)	0.10 (0.06-0.21)	0.11 (0.05-1.16)	0.59 (0.16-2.01)	**.012**
Specific IgE to scallop	2.30 (0.46-9.78)	0.20 (0.13-1.37)	0.13 (0.08-3.39)	0.99 (0.33-4.64)	.059
Specific IgE to cod	1.75 (0.42-6.03)	0.83 (0.22-3.55)	1.45 (0.14-4.11)	1.72 (0.26-6.09)	.099
Specific IgE to tuna	0.82 (0.34-2.44)	0.37 (0.11-1.63)	0.98 (0.09-1.84)	0.92 (0.21-4.53)	.055
Specific IgE to salmon	1.34 (0.28-5.45)	0.82 (0.15-4.72)	1.01 (0.14-4.54)	1.75 (0.43-9.38)	.498
Specific IgE to rCypc1	6.17 (1.30-32.50)	2.54 (0.80-10.85)	5.56 (0.87-21.50)	5.61 (0.85-14.08)	**.041**
Specific IgE to rGadc1	5.06 (1.32-20.00)	1.83 (0.49-8.52)	4.80 (0.67-9.71)	3.74 (0.73-12.96)	**.004**
Others, median (IQR)					
Total IgE (kUA/L)	740 (229-2250)	764 (563-1010)	525 (307-1440)	2598 (484-4096)	.802
SPT to *D.p.* (mm)	6.00 (4.00-8.00)	6.00 (3.50-9.00)	4.25 (0.63-7.63)	6.00 (3.00-9.00)	.560
SPT to *D.f.* (mm)	5.00 (3.00-8.00)	6.00 (3.00-9.00)	4.25 (0.00-8.50)	5.00 (2.50-8.25)	.487

D.f., *Dermatophagoides farina*; *D.p*, *Dermatophagoides pteronyssinus*; *IQR*, interquartile range; *OFC*, oral food challenge; *SPT*, skin prick test.
Significant values ($P < .05$) are bolded.
*Challenge-positive to shrimp but tolerating other crustaceans such as lobster, crab, and crayfish.

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