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Fish collagen as a potential indicator of severe allergic reactions among patients with fish allergies

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Running head: Fish collagen and severe fish allergy

Keywords: fish collagen, anaphylaxis, allergen component, component-resolved diagnostics, ELISA

Conflicts of interest

AY, KS, KF, KK, TY and TH have no conflicts of interest to declare. NS, FO, YT and MN are employee of Hoyu Co.,Ltd. SO receives research funding from Hoyu Co.,Ltd. KM belongs to an endowed department sponsored by Hoyu Co., Ltd.

Author contribution statement

The named authors meet the Clinical & Experimental Allergy criteria for authorship of this paper.

NS, AY and KM were involved in the planning of the study. NS and AY were involved in the search, selection and quality appraisal of included studies. FO, YT and SO performed the experiments and data analysis. NS, KS and KF were involved in the interpretation of results. NS drafted the manuscript. FO developed the methods section with NS. AY, KS, KF, KK and KM reviewed and commented on data analyses and criteria of clinical score. MN, KS, KK, KF, SO, TY, TH and KM reviewed this paper and provided feedback. All authors reviewed and approved the final draft for publication.

Key message:

- We investigated the relationship between symptom severity and IgE reactivity to specific allergens in fish
- We found IgE reactivity to fish collagen, but not parvalbumin, was associated with symptom severity
- Fish collagen-specific IgE may help detect severe fish allergy, but these findings need confirmation.

To the Editor,

Fish allergy, which is usually a lifelong disease, occurs mostly in countries with high fish consumption, including Japan, and presents with mild to severe symptoms, such as oral allergy syndrome, diarrhea, urticaria, dyspnea, and in severe cases, even life-threatening anaphylactic shock (reviewed in Kuehn¹). β -parvalbumin and collagen, which are both well-known fish allergens, are cross-reactive allergens present in various species of fish (reviewed in Kuehn¹).² A few recent case studies reported that fish collagen induced severe allergies³⁻⁵; however, no significant relationship was evident between the severity of the symptoms and the collagen-specific IgE reactivity. In this study, we investigated, for the first time, the relationship between the severity of the symptoms and sensitization to fish collagen in Japanese patients with fish allergy. Additional information about study methods and findings are available in the following repository

[https://urldefense.com/v3/__https://sandbox.zenodo.org/record/921685*.YVQ59prP2Uk__;Iw!!N11eV2iwtfs!9sddhpOKuExU8b03PqsWm2hz7SmNL6z7JM-1w2M3U4QjAtBor4_j21Zv5Q6eYjUT5g\\$](https://urldefense.com/v3/__https://sandbox.zenodo.org/record/921685*.YVQ59prP2Uk__;Iw!!N11eV2iwtfs!9sddhpOKuExU8b03PqsWm2hz7SmNL6z7JM-1w2M3U4QjAtBor4_j21Zv5Q6eYjUT5g$)

This study's protocol was approved by the Institutional Review Board of Fujita Health University (approval number: HM20-204). Written informed consent was provided by all patients or their parents. Detailed information on the materials and methods used are provided in the online supplement (View repository). Briefly, 21 patients (15 females and 6 males; mean age, 26.0 years; range, 10–50 years) who were diagnosed with fish allergy based on their history underwent testing for specific IgE to fish (Thermo Fisher Diagnostics, Phadia AB, Uppsala, Sweden) and/or skin prick tests (SPTs) to fish at Fujita Health University General Allergy Center, Bantane Hospital in Aichi, Japan, in 2017-2020. We performed SPTs according to standard methods⁶ using various

fishes (as is) and extracted collagen (10 and 100 µg/mL). The SPT result was considered positive if the wheal was larger than half of the diameter of the positive control (10 mg/mL histamine dihydrochloride, Torii Pharmaceutical, Tokyo, Japan) or if more than 3 mm was observed after application for 15 min. Patients were assigned a clinical score of their allergy severity on a 5-point scale based on the allergic symptoms reported during the interview, using a slightly modified version of the scoring system developed by Astier et al.⁷ A score ≥ 3 was defined as “severe” (View Repository). There were 5, 6, 5, and 5 patients with clinical symptoms scored as 4, 3, 2, and 1, respectively, based on the intake interview. Eleven patients (numbers 1–11) had a score ≥ 3 and were recognized as “severe,” as they experienced anaphylaxis (Table 1). Fifteen patients (numbers 1–6, 9, 10, 12, 13, 16–20) demonstrated cross- or co-sensitization to two or more fish species as observed on the CAP-FEIA system and SPT. No association was found between the severity of the symptoms and clinical manifestations, such as atopic dermatitis, hand eczema, bronchial asthma, fish-specific IgE, SPT to fishes, or the total IgE level. β -parvalbumin derived from silver salmon was produced in *Escherichia coli* (*E. coli*) using the pET expression system. Natural collagen was extracted from heated silver salmon because collagen contains hydroxyproline, which is a post-translational modification of proline residues in eukaryotes. Prokaryotes, such as *E. coli*, do not have the modification enzyme to convert proline to hydroxyproline. On sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), both proteins showed bands that were consistent with their expected size (View Repository). Native collagen is a heterotrimer composed of two α -1 and one α -2 chains. The electrophoretic patterns in the SDS-PAGE showed three major bands: two bands at 110–160 kDa (a higher and a lower band that corresponded to α -2 and α -1 chains, respectively) and a band at 160–260 kDa consisting of two α chains as a dimer.⁸ To assess IgE reactivity and the contamination of other allergens in the extracted collagen, immunoblot analysis and enzyme-linked immunosorbent assays (ELISA) were performed using sera from all

patients. In the IgE immunoblotting, the patients' IgE reacted with protein bands at 110-160 kDa and 160-260 kDa in the extracted collagen sample (View Repository). IgE-binding to collagen was more frequently detected in patients with score ≥ 3 (P1-9, 9 of 11 [81.8%]) than in patients with score 1 and 2 (P12, P13, P17-19, 5 of 10 [50.0%]). Sera from a non-allergic individual had no IgE reactivity in the same sample. Therefore, highly purified collagen was obtained from heated fish extract. We performed SPT using the collagen extract on patient number 4 as a representative patient; the results showed clinical reactivity to fish collagen (1 $\mu\text{g/mL}$, 3×3 mm). In addition, a positive control test (histamine) showed a 7×7 mm result, whereas a negative control test (physiological saline) showed a negative result (data not shown). In ELISA, every sample with values greater than the mean value of non-allergic individuals plus 5 standard deviations was considered positive. Figure 1 illustrates the relationship between clinical scores for allergic symptoms and the collagen and β -parvalbumin ELISA values. ELISA analysis showed that 11 (52.4%) and 10 (47.6%) of 21 patients with fish allergy were positive for collagen and β -parvalbumin, respectively. The positivity rates for specific IgE against collagen in each clinical score were 80% (4/5) for those with a score of 4; 66.7% (4/6) for a score of 3; 40% (2/5) for a score of 2; and 20% (1/5) for a score of 1. For β -parvalbumin, the positivity rates were 40% (2/5) for a score of 4, 50% (3/6) for a score of 3, 40% (2/5) for a score of 2, and 60% (3/5) for a score of 1. There was a general trend of increasing clinical score with increasing rate of immunopositivity to collagen. Of note, among the 11 patients with severe fish allergy, 8 (72.7%) and 5 (45.5%) were positive for IgE specific to fish collagen and β -parvalbumin, respectively. Therefore, immunoreactivity to fish collagen was effectively detected in patients with severe fish allergy.

In the early 2000s, type I collagen was identified as a fish allergen in Japan.⁸ In the past decade, a few cases of fish collagen-induced allergy have been reported in the literature.³⁻⁵ Notably, all patients in the case reports experienced severe symptoms after intake of fish collagen and/or

heated fish. Recently, the clinical relevance of fish collagen was investigated in a large patient population (n = 101).⁹ The inclusion of relevant collagen allergens in routine diagnosis has shown to improve the ability to accurately diagnose fish allergy. However, there are no published studies with an adequate number of patients with fish allergies and detailed information on their symptom severity. In this study, we focused on the relationship between severity and IgE reactivity against fish collagen, and our results suggest that it may be possible to detect patients with severe fish allergy by measuring collagen-specific IgE.

Although native collagen in raw fish is insoluble, it becomes water-soluble with heat treatment. Sensitivity to fish collagen is under-reported because of solubility problems and false-negative skin test results.¹⁰ SPT, using heated fish extract, is very useful for diagnosing fish collagen-induced allergy. As an alternative, measurement of the specific IgE to fish gelatin (e.g. with CAP-FEIA) may be useful for diagnosing fish allergy if SPT with heated fish extract is not available. Specific IgE to fish gelatin was measured in the three published case reports, and two of three patients tested positive.³⁻⁵ A limitation of this study is that fish collagen sensitivity has been tested using a limited number of fish species, and the cross-reactivity remains unclear. There is an urgent need for more comprehensive studies with a greater number of patients, various fish species against which reactivity is observed, collagen preparation from various fishes, and other fish allergic components, such as enolase, aldolase, and glyceraldehyde 3-phosphate dehydrogenase. In conclusion, we identified fish collagen that can be used as a potential indicator to detect severe fish allergies.

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Figure legend

Figure 1. Enzyme-linked immunosorbent assay for evaluating the IgE reactivity of patients' sera against native collagen (A) and recombinant β -parvalbumin (B). The grey bars indicate the patients and light grey bars indicate the non-allergic individuals. The threshold for positivity (dashed line) was calculated using the average signal from 8 non-allergic individuals + 5 standard deviations: nCol, native collagen; rParv, recombinant β -parvalbumin; O.D., optical density.

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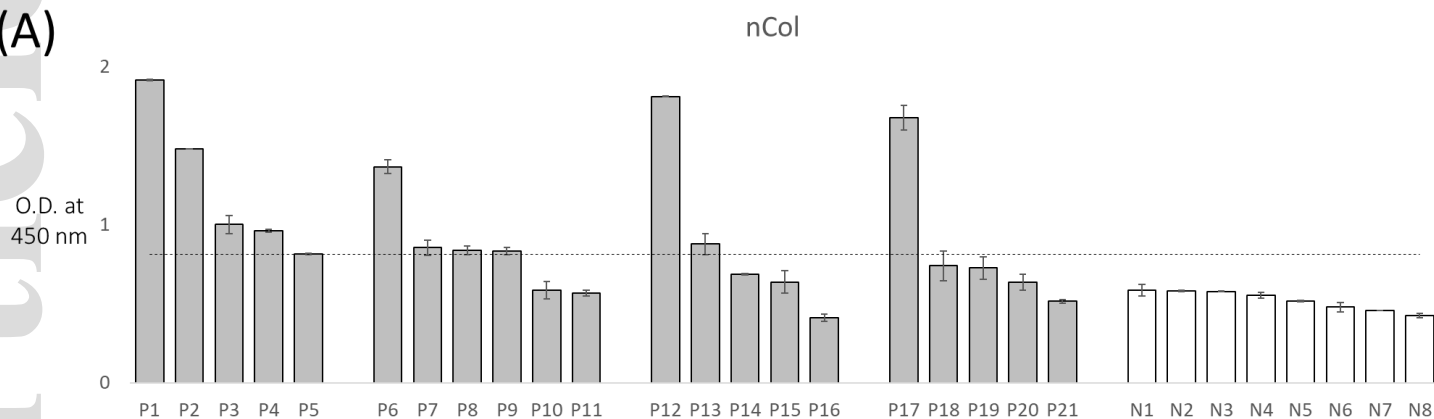
Table 1. Clinical data of patients with fish allergy

Patient no.	Clinical score	Age at onset (y)	Sex	Clinical manifestations			Total IgE	Specific IgE (positive fish)	Skin prick test (positive fish)	Clinical history of fish allergy
				AD	Hand eczema	BA				
1	4	39	F	+	+	-	1778	cod, salmon, tuna	eel	eel: Dy, C, U, Ap
2	4	19	F	-	+	+	52	flounder, salmon, tuna	eel, flounder, mackerel	rockfish: Dy, Ht, U eel, flounder, mackerel, salmon, tuna: U
3	4	16	F	-	+	+	465	cod, mackerel	eel, flounder, mackerel, salmon sea bream, yellowtail	eel: Dy, Ap, PhaS, U salmon, sea bream: Ap, PhaS
4	4	42	F	+	+	+	590	-	sea bream, sweetfish	sea bream, sweetfish: Ht sardine: OAS
5	4	20	M	-	+	-	841	cod, flounder, mackerel, salmon, sardine, tuna	eel, flounder, mackerel, salmon, yellowtail	cod, flounder, mackerel, salmon, tuna: Dy, PhaS, Ap, OAS
6	3	27	M	+	+	+	1740	salmon	rockfish	rockfish: ReD, FaS
7	3	14	F	-	-	-	278	-	flounder	flounder, yellowtail: Ap, U, OAS
8	3	38	F	-	-	-	562	-	flounder	cod, flounder: ReD, Ap
9	3	15	F	-	-	-	159	-	barracuda, flounder	barracuda, flounder: V, Ap, U
10	3	27	F	+	+	-	356	cod, flounder, horse mackerel, mackerel, salmon, sardine, tuna	flounder, rockfish, salmon	rockfish, salmon: ReD, OAS
11	3	49	F	+	+	-	75	-	tuna	tuna: ReD, U, FaS
12	2	17	F	+	+	-	2300	salmon, tuna	flounder, salmon, tuna	tuna: ReD
13	2	21	F	-	-	-	1136	cod, horse mackerel, salmon sardine	atka mackerel, herring, mackerel, smelt	atka mackerel, herring: U, OAS
14	2	22	F	+	+	-	840	-	mackerel	mackerel, pacific saury: U
15	2	28	M	-	-	-	709	-	yellowtail	flounder: U
16	2	20	F	-	-	-	134	-	flounder, yellowtail	flounder, yellowtail: FaS
17	1	30	M	+	+	+	2912	-	salmon, small fish, tuna	,eel, horse mackerel, mackerel, rockfish, salmon: Di, V
18	1	18	M	+	+	-	8010	cod, flounder, mackerel, salmon, tuna	yellowtail	cod, salmon, yellowtail: C, U
19	1	10	M	-	-	-	559	cod, flounder, horse mackerel, mackerel, salmon, sardine, tuna	horse mackerel, mackerel	horse mackerel: U
20	1	24	F	+	+	+	251	-	salmon, sea bream	salmon: FaS
21	1	50	F	-	-	-	781	-	smelt	mackerel: Ap, smelt: Ap, Di

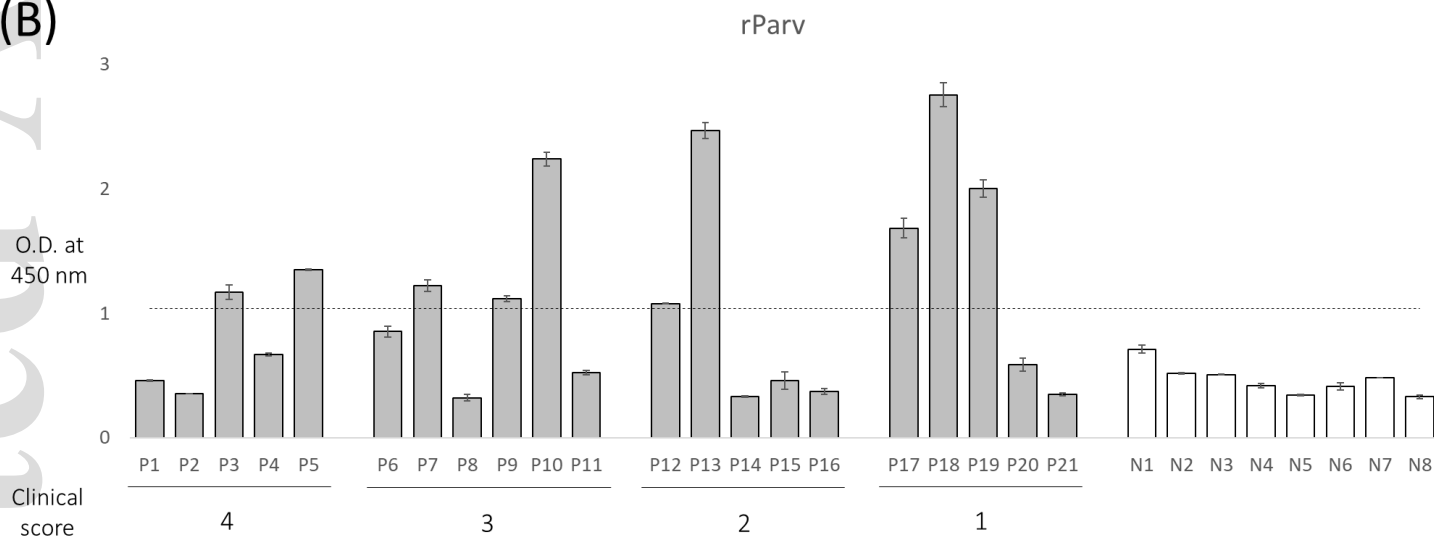
M, male; F, female; Y, years; Symptoms: AD, atopic dermatitis; BA, bronchial asthma; Ap, abdominal pain; C, cough; Di, diarrhea; Dy, dyspnea; FaS, facial swelling; Ht, hypotension; OAS, oral allergy syndrome; PhaS, pharyngeal swelling; ReD, respiratory discomfort; U, urticarial; V, vomiting. Specific IgE, determined using the CAP-RAST system (Thermo Fisher Diagnostics). Clinical scores were defined as described in the online supplement.

Figure 1.

(A)



(B)



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