



Letter

Correlation of clinical monosensitivity to cod with specific IgE to enolase and aldolase

Fish can induce allergic reactions in sensitized persons. Symptoms occur not only on ingestion of fish but also on inhalation of cooking vapors or contact with fish.¹ Symptoms range from oral pruritus, urticaria, angioedema, and asthma to systemic anaphylaxis in some cases.^{2,3} Fish allergic patients have a risk of approximately 50% of experiencing allergic cross-reactions to other fish species.⁴ IgE binding to parvalbumin, the major cross-reactive fish allergen, has been characterized as a hallmark for clinical cross-reactivity in fish allergy.^{1,5} However, monosensitivity to salmonid fishes related to IgE to single parvalbumin isoforms of salmon and trout can occur.^{6,7} Monosensitivity or oligosensitivity to specific fishes has been reported in a few publications that describe patients with no IgE binding to parvalbumin but IgE binding to other allergens.^{1,8} Recently, we identified patients sensitized to parvalbumin from cod, salmon, and/or tuna, which were cosensitized to enolases in 31 of 45 patients (69%) and to aldolases in 24 of 45 patients (53%).⁹ Parvalbumin-negative patients ($n=17$) had specific IgE antibodies to enolases in 47% and to aldolases in 41% of study participants.

In the present study, we report the sensitization pattern of 3 patients who repeatedly experienced moderate to severe allergic reactions on ingestion of fish belonging to single species while they tolerated ingesting other fish species (eTable 1).

Patient 1, a 39-year-old man, had swelling of the lips and tongue, throat discomfort, stomach pain, and diarrhea on consumption of cod and Nile perch while he tolerated other fishes. Skin test results were positive for fresh cod and Nile perch but negative for other fishes (salmon, trout, tuna, mackerel, and herring). In vitro IgE test results were negative for cod (<0.1 kU_A/L) (Phadia ImmunoCAP system; ThermoFisher Scientific, Waltham, Massachusetts). Patient 2, a 10-year-old boy, reacted with swelling of the lips and tongue, throat discomfort, and conjunctivitis repeatedly after eating cod while he tolerated salmon. Skin prick test results revealed a strong sensitization to cod but were negative for salmon. The specific IgE level was 0.4 kU_A/L for cod, but test results were negative for salmon and tuna. Patient 3, a 48-year-old man, experienced generalized urticaria and angioedema on consumption of cod and mackerel. He tolerated salmon, seabream, and swordfish. Skin test results were positive for cod, salmon, tuna, and herring and indeterminate for mackerel. The specific IgE levels were 0.1 kU_A/L for cod and salmon and 1.5 kU_A/L for tuna. All 3 patients refused oral challenge, with the fish species provoking clinical symptoms.

Fish extracts were prepared from cod, salmon, trout, tuna, and Nile perch, and IgE immunoblots were performed as described elsewhere (sera dilution 1:3).⁹ The serum of patient 1 revealed 2 major bands in cod extract at 40 to 50 kDa identified as aldolase and

enolase (20-kDa band identified as enolase degradation product by Edman sequencing) and in Nile perch extract at 40 to 50 kDa but not in other fish extracts (eFig 1; salmon and tuna not shown). Patients 2 and 3 had IgE binding to 2 cod proteins at 40 to 50 kDa (20 kDa, enolase degradation product) only. Parvalbumin isoform mixtures, enolases, and aldolases were purified from cod, salmon, tuna, and Nile perch (eFig 2).⁹ IgE enzyme-linked immunosorbent assay (ELISA) (sera dilution 1:3, 1:4, and 1:5) was performed as described previously.^{9,10} For all patients, specific IgE titers were found for cod enolase and aldolase (Fig 1; salmon and tuna not shown). Patient 1 had also specific IgE to Nile perch enolase and aldolase. IgE immunoblot inhibition was performed for patients 1 and 3. Addition of cod enolase and aldolase abolished IgE binding to Nile perch (patient 1) and mackerel (patient 3) proteins, strongly suggesting a primary sensitization to cod for both patients (eFig 3). This was confirmed by ELISA for patient 1, the results of which revealed that inhibition with cod allergens (maximal inhibition, 92%–97%) was efficient, whereas Nile perch homologs gave only a weak inhibition (eFig 4).

For 2 patients (patients 1 and 2), there was a good correlation between clinical history and skin prick tests. For patient 3, clinical symptoms and skin tests were less correlated. A positive correlation of the clinical history and in vitro IgE reactivity (ImmunoCAP) was only found for patient 2. ImmunoCAP results for patients 1 and 3 were negative (<0.1 kU_A/L) for cod extract. Overall, the results from our in vitro IgE determinations using purified allergens correlated with the clinical sensitivity of the patients. Only enolases and aldolases were detected by both immunoblot and ELISA. Although fish enolases and aldolase share a high interspecies protein identity, IgE reactivity to species-specific epitopes of enolase and aldolase seems to be present in these patients. Previously, we found IgE antibodies to these allergens in 38% of patients with monosensitization or oligosensitization to specific fishes but no direct correlation between specific IgE binding and the clinical history.⁹ We report, for the first time to our knowledge, a correlation of specific IgE reactivity to fish enolases and aldolases with the clinical history of species-specific sensitization in fish allergic, parvalbumin-negative patients. The present study suggests that the use of a panel of fish allergens (parvalbumins, enolases, and aldolases) could be of added value for component-resolved diagnosis of fish allergy. Single allergen-specific IgE reactivity might be associated with clinical reactivity to specific fishes.

All our patients declined oral provocation tests with fish. Because they had repeatedly experienced allergic symptoms when consuming the culprit fish species, the clinical histories were convincing enough to conclude that these patients are primarily sensitized to cod with serologic and clinical cross-reactivity to Nile perch and mackerel, respectively. A food challenge would confirm the clinical monosensitivity, but it would not be helpful to identify the responsible allergen because fish muscle contains several

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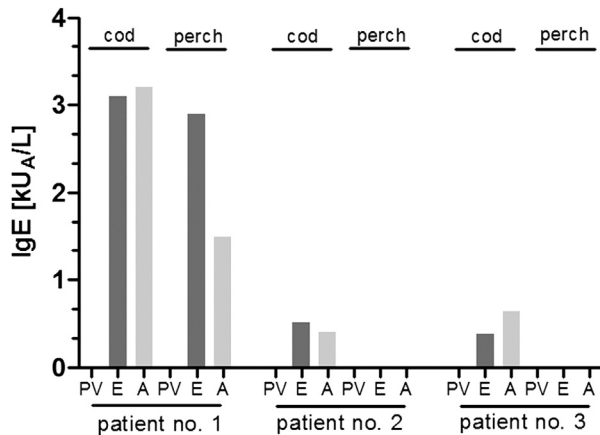


Figure 1. IgE quantification by enzyme-linked immunosorbent assay. Cod enolase (E) and aldolase (A) were detected by IgE from patients 1 through 3. Patient 1 had additional IgE-reactivity to Nile perch enolase and aldolase. PV indicates parvalbumin.

allergens, parvalbumins, enolases, aldolases, and other, still unidentified allergens.

Even though most fish allergic persons experience allergic reactions to multiple fish species,^{1,2,4} some individuals are sensitized to single or a limited number of fishes only. Commercial in vitro IgE tests do not seem to be helpful in establishing a correct and sensitive diagnosis in individuals with species-specific fish allergy. In these patients, sensitization to fish enolase and aldolase in addition to parvalbumin isoforms has to be considered.

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Supplementary Data

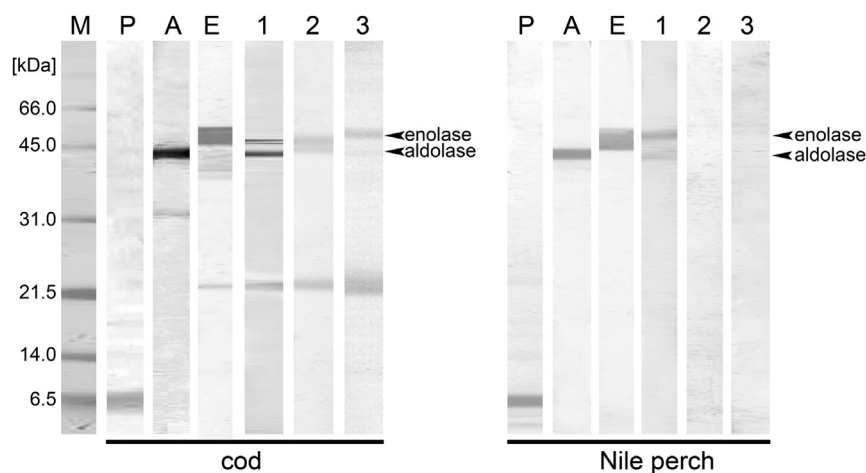
Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.09.005>.

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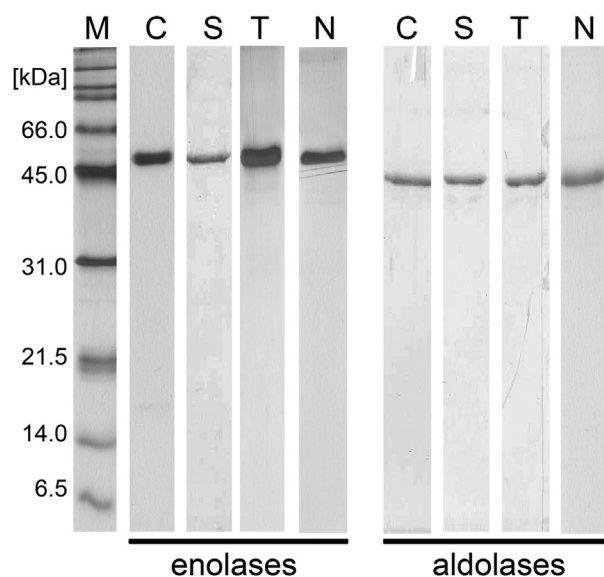
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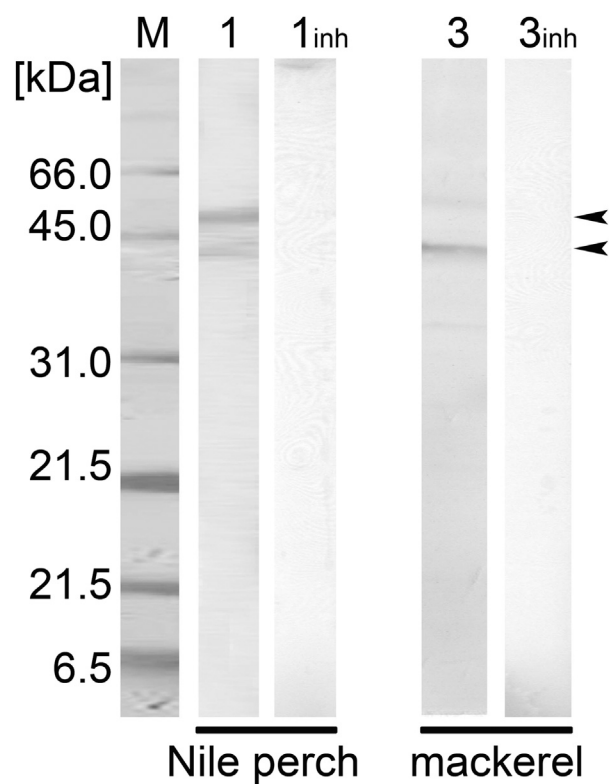
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eFigure 1. IgE detection by immunoblot. Cod allergens (40–50 kDa; 20 kDa, degraded enolase) were detected by cod-sensitized patients 1 through 3 in total extract. Nile perch allergens (40–50 kDa) were recognized by Nile perch-sensitized patient 1 only. M indicates marker; P, parvalbumin; A, aldolase; and E, enolase.



eFigure 2. Purity of fish allergens from cod (C), salmon (S), tuna (T), and Nile perch (N). Enolases and aldolases were purified by chromatography⁹ to homogeneity as visualized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and silver stain. M indicates marker.



eFigure 3. Cross-inhibition of IgE reactivity to Nile perch and mackerel extract. Serum samples from patients 1 and 3 were efficiently inhibited with 100 $\mu\text{g/mL}$ of each cod enolase and aldolase. M indicates marker; inh, inhibited.

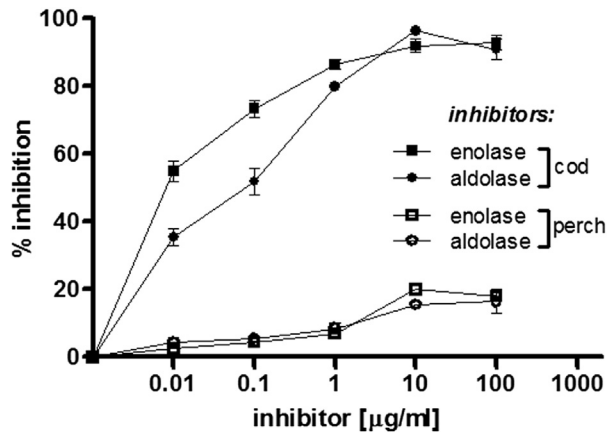


Figure 4. IgE cross-inhibition of cod and Nile perch allergens. IgE inhibition of serum sample from patient 1 by cod enolase/aldolase (0.01–100 µg/mL) was strong, whereas Nile perch allergens were weak inhibitors.

eTable 1

Patient characteristics by clinical routine diagnosis

Patient No.	Sex/Age, y	Reactivity	Symptoms	SPT	Total IgE, ^a kU/L	Specific IgE, ^a kU _A /L
1	M/39	Cod, Nile perch	AP, D, OAS	Positive: cod, Nile perch Negative: salmon, tuna	80.3	Positive: none Negative: cod
2	M/10	Cod	E, OAS	Positive: cod Negative: salmon, tuna	520	Positive: cod 0.4 Negative: salmon, tuna
3	M/48	Cod, mackerel	AE, E, U	Positive: cod, salmon, tuna Negative: mackerel	973	Positive: cod 0.1, salmon 0.1, tuna 1.5 Negative: none

Abbreviations: A, asthma; AE, angioedema; AP, abdominal pain; D, diarrhea; E, eczema; OAS, oral allergy syndrome; SPT, skin prick test; U, urticaria.

^aIgE determination by ImmunoCAP: negative was considered a level lower than 0.1 kU_A/L; positive was considered a level of 0.1 kU_A/L or greater.