Allergy To *Limanda aspera* (Yellowfin Sole): Report of a Case of Food Allergy in a Child

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Fish allergy is one of the most common food allergies in Spain because fish is a major part of the diet in the Mediterranean area. Despite broad biodiversity, the most frequently consumed species belong to a limited number of orders, namely, salmon-like (Salmoniformes), cod-like (Gadiformes), perch-like (Perciformes), herring-like (Clupeiformes), carp-like (Cypriniformes), catfish-like (Siluriformes), and flatfish (Pleuronectiformes) [1]. The market share of these species varies from country to country according to regional availability and eating habits [1], which are constantly changing.

One example of new consumption is the white fish yellowfin sole (*Limanda aspera*), a flatfish of the *Pleuronectidae* family that lives on soft seafloor at depths of up to 700 meters and whose native habitat is the temperate waters of the northern Pacific [2]. Yellowfin sole is consumed regularly in Japan, Korea, and Canada. However, nowadays, globalization of the fishing trade and the high price of native species favor replacement by other species such as yellowfin sole [1,2].

Most fish-allergic patients present symptoms to a range of fish species, mainly owing to sensitization to the fish panallergen parvalbumin [3,4]. Parvalbumins are low-molecular-weight proteins that are highly conserved across several fish species [3], although fish allergens other than parvalbumin have been reported, for example, aldehyde phosphate dehydrogenase (41 kDa) from codfish and transferrin-like protein (94 kDa) from tuna [5]. In addition, fish proteins such as enolases, aldolases, and fish gelatin have been identified as major new fish allergens [4-6].

No data on food allergy to yellowfin sole have been published. Therefore, we analyzed the IgE-binding profile of a fish-allergic child who was sensitized exclusively to *Limanda aspera*.

A 2-year-old white boy developed facial urticaria immediately after eating or touching yellowfin sole. He tolerated other fish (eg, sole, cod, monkfish, hake, and tuna) by ingestion and even contact. His personal history of atopy included allergy to nuts, which presented as acute urticaria after ingestion of hazelnuts and almonds.

Skin prick tests were performed with *Anisakis simplex* and commercial fish extracts (Leti) of tuna, cod, hake, sole, sea bream, red mullet, sardine, and roosterfish. Prick-by-prick testing was performed with yellowfin sole. Histamine hydrochloride 10 mg/mL and 0.9% saline were used as positive and negative controls, respectively. Total IgE and specific IgE to sole extract and recombinant parvalbumin from cod (rGad c 1) were determined using the ImmunoCAP system (Thermo Fisher Scientific). IgE levels of more than 0.35 kU_A/L were considered positive.

Extracts from yellowfin sole, tuna, cod, hake and sole were prepared from raw commercial fish (La Sirena Alimentación Congelada). *Anisakis* was collected from hake. All the species were cut into small pieces, ground, and suspended in Coca solution (NaCl 10 mg/mL, $NaHCO_3$ 4.34 mg/mL, Tween 20 0.4%) for 2 hours at 4ºC. After centrifugation, the supernatant was discarded, and the pellet was resuspended in Coca solution for 2 hours at 4ºC. Supernatants were dialyzed and later lyophilized. The protein concentration was determined according to Bradford [7]. SDS-PAGE and Western blot were performed as described elsewhere [8,9].

Briefly, fish extracts were separated by SDS PAGE under reducing conditions in MiniPROTEAN II gel (Bio-Rad) before being transferred to polyvinylidene difluoride membranes. The membranes were incubated all night with the patient's serum (dilution 1:5), washed, and then treated with IgE mouse antihuman IgE Fc-HRP (dilution 1:1000 2 hours) (Southern Biotech). IgE-binding components were detected using chemiluminescence according to the manufacturer's instructions (PerkinElmer).

Skin prick tests were negative for all commercial fish extracts and for *Anisakis simplex*. Prick-by-prick testing was negative with cooked yellowfin sole but positive with raw yellowfin sole, revealing a clear wheal of 5 mm (histamine control, 3 mm). Total IgE was 761.6 IU/mL. Specific IgE for rGad c 1 was negative (0.27 kU_A/L). In the immunoblot analysis of total fish extracts, the patient's IgE detected 2 bands of about 35 kDa and 37 kDa in yellowfin sole extract. No signals were obtained for protein extracts from tuna, cod, sole, *Anisakis simplex*, or hake (Figure).

Parvalbumins are recognized as major fish allergens that cause IgE cross-reactivity between fish species [3,6].

We studied a child with clinical symptoms due exclusively to yellowfin sole. IgE analysis (ImmunoCAP and Western

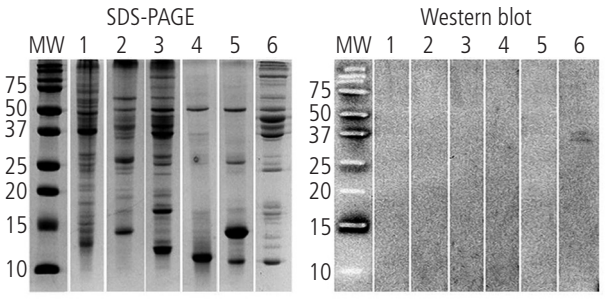

Figure. SDS-PAGE and Western blot. MW indicates molecular weight. Lane 1, *Anisakis*; lane 2, tuna; lane 3, cod; lane 4, hake; lane 5, sole; lane 6, *Limanda aspera*.

blot) showed that fish parvalbumins were not the responsible allergens.

Sensitization to a single fish species caused by allergens other than parvalbumin has already been reported [5,6,10, Supplementary reference 11]. We observed the presence of 2 proteins of about 35 kDa and 37 kDa whose identity remains to be determined, although according to the studies of Kuehn et al [Supplementary reference 12] they could correspond to an aldolase.

To date, only occupational asthma has been reported in workers exposed to aerosolized yellowfin sole [Supplementary reference 13]. Yellowfin sole is one of the most abundant flatfish species in the eastern Bering Sea and is the largest flatfish caught in the United States. Workers live on factory ships, where they sort, process, and freeze the harvested fish. Some of these workers have reported asthma-like symptoms, although only 6% were sensitized to fish species [Supplementary reference 14].

In summary, we report the case of a child sensitized to yellowfin sole but not to other species of fish. Western blot identified 2 proteins with molecular weights of 35 and 37 kDa in *Limanda aspera*. Both bands could correspond to a new fish allergen family, although further characterization is necessary.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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