

Selective allergy to whiff (*Lepidorhombus whiffiagonis*): identification of enolase as a new major allergen

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Allergy to fish, one of the most common food allergies, is generally IgE-mediated and tends to elicit severe reactions [1]. In many cases, polysensitization and allergy to multiple species is explained by cross-reactivity with parvalbumin, a major fish allergen [2]. However, some patients are allergic only to specific species while tolerating other types of fish in which parvalbumins [3], allergens other than parvalbumins [4, 5], and co-sensitization to parvalbumin and enolases/aldolases have been implicated [6]. To date, studies have investigated monosensitivity to sole, swordfish, pangasius/tilapia, tuna/marlin, cod, salmon, and conger [7-12]. Although hake is the most widely consumed fish species in Spain, sole (*Solea solea*) and whiff (*Lepidorhombus whiffiagonis*), which belong to the Scophthalmidae family, have increased considerably in popularity over the last 3 years

(https://eumofa.eu/documents/20178/415635/ES_El+mercado+pesquero+de+la+UE_2020.pdf).

β -parvalbumin (Lep w 1, 11.5 kDa) is the only allergen identified in whiff to date. However, allergens such as Sol so 1 (parvalbumin) and Sol so 8 (triosephosphate isomerase) have been described in sole (WHO/IUIS; www.allergen.org). In routine clinical practice, we frequently recommend that patients avoid sole after developing an allergic reaction to whiff if their allergic status to sole is unknown.

We aimed to study serum IgE reactivity in fish allergic patients with previous allergic reactions after ingesting whiff who tolerated consumption of fish species not related to

order pleuronectiformes. Inclusion criteria were: 1) Subjects with a clear history of whiff allergy and 2) positive skin prick test (SPT) and/or positive serum-specific IgE (sIgE) to whiff extract. This group of patients had developed no allergic reaction on eating fish species not related to order pleuronectiformes. The patients underwent oral challenge tests with salmon, cod, tuna and hake as published elsewhere [13].

Fresh whiff was purchased from a local market. Whiff extracts were prepared from raw and cooked fish as previously described [14]. SPT was performed with the following 9 commercially available fish extracts (Roxall®, Spain) in addition to whiff: anchovy, cod, hake, salmon, sole, swordfish, sardine, trout, and tuna. sIgE to individual species was measured using the Siemens Immulite 2000/Xpi® immunoassay analyzer (Erlangen, Germany).

Ten whiff-allergic patients participated, all of whom tolerated fish species belonging to another order (salmon, cod, hake, and tuna) on the oral challenge.

Sole allergy in this study was considered an exception, as both whiff and sole are species of the same order pleuronectiformes, and it was assumed that the homology between their proteins can be very high. So, an oral challenge test to sole, a fish belonging to the same order Pleuronectiformes than whiff, was considered subsequently to evaluate tolerance in this special group of whiff allergic patients.

Out of the 10 patients included, a sole challenge test was only performed in 4, which was positive in one (n.8) and negative in 3 (n.3, 7, and 9). The remaining patients refused the oral challenge test to sole.

The mean patient age was 18.7 years (1-52, IQR=8.7-25) and 50% were male. Mean age of onset of whiff allergy was 12.7±16.2 years, with urticaria (60%) being the most frequent symptom. Allergic comorbidities including atopic dermatitis and rhino-

conjunctivitis were present in 20% of patients, while 30% presented asthma and 60% another food allergy.

SPT to commercial fish extracts, excluding whiff and sole, were all negative. SPT and specific IgE to whiff and other fish species are shown in the Table of this article's Online Repository.

IgE reactivity to whiff was evaluated by SDS-PAGE IgE-immunoblotting using sera from all patients and raw/boiled whiff extracts obtained following a previously described method [14]. Sera from patients 2, 7, 8, and 9 revealed a similar IgE-binding pattern of ~12-15, 25-30, and ~50-kDa both in raw and boiled extracts. Serum sample 1 only showed a 50-kDa IgE binding band. All other sera showed no IgE-reactive protein in the whiff extract (Figure). Of note, the 50-kDa band was the only one that bound to specific IgE in at least 50% of the patients' sera, suggesting it is the major allergen in selective whiff allergy.

Proteins were identified in the Complutense University of Madrid Proteomic Department by searching a nonredundant protein sequence database (National Center for Biotechnology Information) using the Mascot program (<http://www.matrixscience.com>). When compared against the databases, MS/MS analysis of the resulting peptides corresponded to an enolase-like protein for the 50-kDa band with 43% coverage.

Parvalbumins are small (10-12 kDa) calcium-binding proteins that are resistant to enzymatic digestion and heat [1]. Parvalbumin can be detected as a 12-kDa monomer or as a dimer (24kDa), trimer (36-kDa), or polypeptide of more than 40-kDa, showing remarkable IgE reactivity [14]. Abundant in fish muscle, enolase is a ~50-kDa enzyme involved in glucose metabolism and is less stable than parvalbumin [1]. In our study, this

low stability is reflected in its lower capacity to bind to IgE in the cooked whiff compared to the crude extract (observe in Figure the decreased band intensity in the cooked extract).

The interspecies cross-reactivity of enolase is limited and clearly lower than that of parvalbumins, and enolase-specific IgE more clearly verifies primary sensitization to certain fish species than parvalbumin sIgE [6]. Kuehn et al estimated a 63% prevalence of fish allergy due to enolase [6]. In the population studied here, the rate of whiff enolase sensitization was 50%, causing it to be considered a major allergen.

The low quantity or total absence of enolases in commercial extracts could explain the lack of correlation between the degree of skin reactivity and the allergen sIgE levels in individuals with species-specific fish allergy [6, 15].

In summary, this study provides evidence of selective whiff allergy (with or without sole allergy) characterized by severe allergic reactions after eating whiff. Patients studied were found to be sensitized to whiff and/or sole fish species through parvalbumin and/or enolase although they had no sensitization to other fish species and tolerated these foods. However further studies are needed to investigate the association between sole and whiff allergy.

This selective allergy was seen in the exclusive sensitization to one or both allergens. These findings will have important clinical consequences for both allergists and patients, since the risk of conducting a challenge test to other fish species is low, which will likely facilitate the management of fish allergy.

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Conflicts of interest

All authors declare that there is no conflict of interest.

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FIGURE LEGENDS

Figure. SDS-PAGE immunoblotting. Extracts from raw whiff (A) and boiled whiff (B). Lanes 1-10: patients' serum; Lane NA: control serum (pool of sera from nonatopic subjects); Lane M: molecular mass standard; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

