Selective Allergy to Whiff (*Lepidorhombus whiffiagonis*): Identification of Enolase as a New Major Allergen

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Fish allergy is one of the most common food allergies. It is generally IgE-mediated and tends to elicit severe reactions [1]. In many cases, polysensitization and allergy to multiple species are 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 cosensitization 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 to whiff who tolerated consumption of fish species not related to the order Pleuronectiformes. The inclusion criteria were a clear history of whiff allergy and a positive skin prick test (SPT) and/or positive serum specific IgE (sIgE) result to whiff extract. This group of patients did not experience allergic reactions on eating fish species not related to 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 whiff and the following 9 commercially available fish extracts (Roxall): anchovy, cod, hake, salmon, sole, swordfish, sardine, trout, and tuna. sIgE to individual species was measured using the Siemens Immulite 2000/Xpi immunoassay analyzer (Siemens).

The study population comprised 10 whiff-allergic patients, all of whom tolerated fish species belonging to another order (salmon, cod, hake, and tuna) in the oral challenge.

Sole allergy in this study was considered an exception, as both whiff and sole belong to the same order (Pleuronectiformes) and it was assumed that the homology between their proteins was very high. Therefore, an oral challenge test with sole, which also belongs to Pleuronectiformes, was subsequently proposed to evaluate tolerance in this special group of whiff-allergic patients.

A sole challenge test was performed in only 4 of the 10 patients included. The result was positive in 1 patient (patient 8) and negative in 3 patients (patients 3, 7, and 9). The remaining patients refused the oral challenge test to sole.

The mean (SD) age was 18.7 years (1-52 [IQR, 8.7-25]), and 50% of patients were male. Mean age at onset of whiff allergy was 12.7 (16.2) years, with urticaria (60%) being the most frequent symptom. Allergic comorbidities including atopic dermatitis and rhinoconjunctivitis were present in 20% of patients, while 30% presented with asthma and 60% another food allergy.

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

IgE reactivity to whiff was evaluated using SDS-PAGE IgE-immunoblotting with sera from all the 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. Only serum sample 1 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 sIgE 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

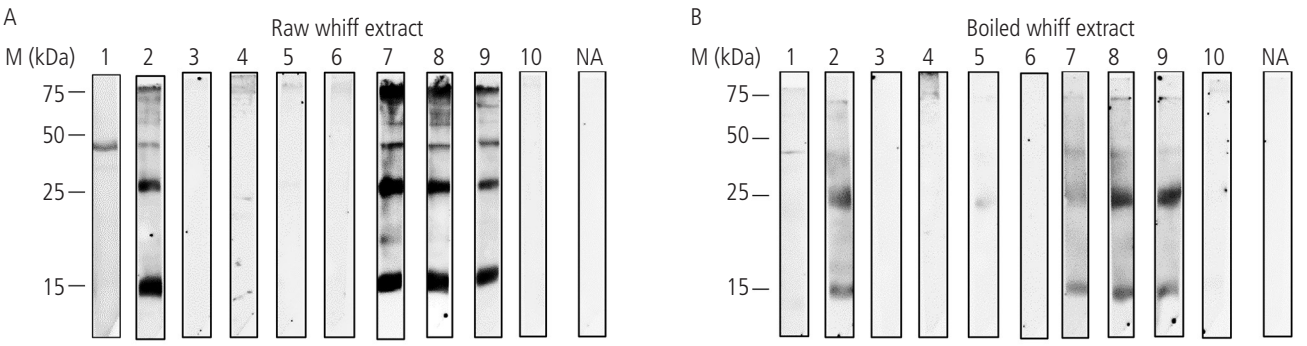

Figure. SDS-PAGE immunoblotting. Extracts from raw whiff (A) and boiled whiff (B). Lanes 1-10, patients' serum; Lane NA, control serum (pool of sera from nonatopic individuals); Lane M, molecular mass standard.

Information) using the Mascot program (http://www.matrixscience.com). When compared against the databases, MS/MS analysis of the resulting peptides revealed 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 monomer (12 kDa), as a dimer (24 kDa), as a trimer (36 kDa), or as a polypeptide (>40 kDa) and shows 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 than in the crude extract (see the decreased band intensity in the cooked extract in the Figure). 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 [6] estimated a 63% prevalence of fish allergy due to enolase. In the population studied here, the rate of sensitization to whiff enolase was 50%, leading 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. The patients studied were sensitized to whiff and/or sole species through parvalbumin and/or enolase, although they were not sensitized to other fish species, which they tolerated. However, further studies are needed to investigate the association between sole and whiff allergy.

This selective allergy was seen in the exclusive sensitization to 1 or both allergens. These findings will have important clinical consequences for both allergists and patients, since the risk of challenge testing with other fish species is low, thus likely facilitating the management of fish allergy.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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