Allergy to Red Mullet and Sea Bass: Two Newly Identified Red Mullet Allergens

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Fish consumption has decreased in the last few years owing to rises in prices throughout Europe. However, Spain remains the largest fish consumer in Europe (https://eumofa.eu/documents/20124/35668/EFM2023_EN.pdf/95612366-79d2-a4d1-218b-8089c8e7508c?t=1699541180521). Red mullet (*Mullus barbatus*) and sea bass (*Dicentrarchus labrax*) are gadiform fish (order Gadiformes) belonging to the bony fish category. Research indicates a greater frequency of fish allergies in regions with a significant consumption of fish and seafood [1-2]. Although the exact prevalence of fish allergy in Spain remains elusive, 11.4% of patients who visited the allergologist did so for the first time, as reported in the most extensive observational study in Spain [2]. Moreover, fish has been reported to be one of the foods most frequently associated with anaphylactic reactions [2].

The primary allergen in fish allergy is ß-parvalbumin. However, individuals diagnosed with specific fish allergy might tolerate other fish species [3]. Using in vitro techniques, we identified 2 red mullet allergens for the first time. Interestingly, only parvalbumin, enolase, and aldolase have been identified as allergens in patients who had experienced hypersensitivity reactions to Gadiformes [3-4].

We report the case of a 54-year-old woman with T2-high allergic bronchial asthma resulting from sensitization to pollens, mites, and animal dander. She began to experience episodes of dizziness, malaise, nausea, vomiting, and diarrhea within 30 minutes after eating grilled sea bass, a fish she had never previously ingested. Subsequently, these symptoms manifested when she ate sea bass. Fourteen months after this episode, she ate fried red mullet for the first time and immediately developed facial erythema with wheals and

eyelid edema. For both reactions, her symptoms subsided 2 hours after administration of dexchlorpheniramine and corticosteroids. No other family member who ate the same fish species had similar symptoms. Since then, she has avoided red mullet and sea bass. The patient tolerates salmon, mackerel, perch, hake, whiting, shrimp, tuna, sardine, and other gadiform fish such as cod and haddock.

In the allergology study, skin prick-prick tests were positive for raw red mullet (5 mm) and for raw sea bass (7 mm) (Supplementary material: Figure 1a) and negative for grilled mullet (0 mm) and grilled sea bass (0 mm). We obtained written consent from the patient to publish her case data. Skin testing was not performed with other species of fish, since the patient tolerated them. The negative control (saline solution) was 0 mm and the positive control (histamine, 10 mg/mL) was 5 mm. The result of blood testing for total IgE was 1126 kU_A/L (UniCap System, Phadia). In the case of specific IgE (sIgE), the results were negative for hake (0.04 kU_A/L), sole (0.12 kU_A/L), and megrim (0.06 kU_A/L). Specific IgE against *Anisakis simplex* and salmon was negative (0.11 kU_A/L and 0.07 kU_A/L respectively). Other potentially relevant sIgE determinations were performed (pollens: platanus, rPhlp1-5b; dust mites: *Dermatophagoides pteronyssinus*; and dog dander) (Supplementary material: Figure 1b).

Protein extracts of grilled mullet, grilled sea bass, raw mullet, and raw sea bass were prepared by homogenization in phosphate-buffered saline followed by centrifugation, dialyzation, and lyophilization. SDS-PAGE IgE immunoblotting assays with the patient's serum were performed under nonreducing conditions (without 2-mercaptoethanol) and showed IgE reactivity with 3 bands in raw mullet and 1 band in raw sea bass (Figure). These IgE-binding proteins were extracted manually from the Coomassie blue gel without 2-mercaptoethanol, digested with trypsin, and analyzed by matrix-assisted laser desorption/ionization mass spectrometry. In order to identify the proteins, we entered their sequence into a protein sequence database (National Center for Biotechnology Information) using the Mascot program. Based on database comparisons, the peptides corresponded to parvalbumin ß-1 (20 kDa) (both raw mullet and raw sea bass), glyceraldehyde-3-phosphate dehydrogenase (27 kDa), and 2-phospho-D-glycerate hydro-lyase (50-75 kDa).

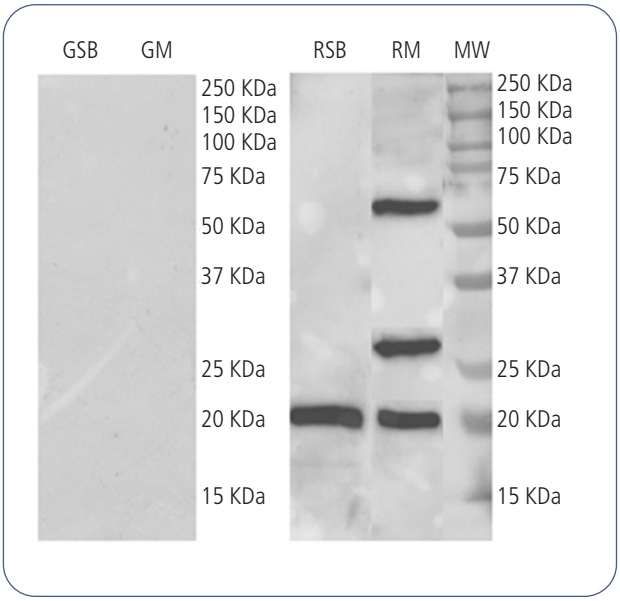

Figure. Detection of reactive proteins to IgE from a fish-sensitive patient's serum. Western blot of raw red mullet (RM), raw sea bass (RSB), grilled red mullet (GRM), and grilled sea bass (GSB) extracts developed with serum IgE. MW indicates molecular weight.

Information about the natural progression of fish allergy remains limited, mostly indicating sustained clinical reactivity in the long term [4]. Fish allergies typically persist throughout life for most individuals, requiring continuous avoidance [4]. Nevertheless, clinical expression of this sensitization may vary, ranging from patients who tolerate most foods despite being strongly sensitized to those who only experience reactions in the presence of cofactors. In clinical practice, determination of sIgE is the most common first diagnostic approach after a complete history when fish allergy is suspected [5-6].

Up to 83 fish allergens of known sequence have been described to date, with most belonging to the parvalbumin family, which is found in most cases of cross-reactivity [7]. In our study, the results of skin prick test and immunoblot were positive with the fish tested in the raw form, and the patient tolerated other gadiform fish such as cod and haddock, in which parvalbumin is the main allergen, indicating that for this patient, the other 2 allergens added to parvalbumin are clinically relevant in the case of red mullet allergy. Selective allergy has been reported to other fish species, such as sole, tuna, cod, salmon, and, recently, whiff [8], owing to cosensitization to parvalbumin and other allergens, such as aldolases and enolases. These apparently less frequent but relevant allergens are less stable than parvalbumin because of their lower capacity to bind to IgE in the cooked form than in the raw extract [8].

Although allergy to fish may cause potentially fatal hypersensitivity reactions, there is a lack of other commercially available fish allergens for the diagnostic work-up, thus reinforcing the suitability of using advanced in vitro techniques, such as SDS-PAGE and mass spectrometry, which may be of help in unveiling unknown allergens [9]. In the present study, we identified 2 new allergens in red mullet, namely, glyceraldehyde-3-phosphate dehydrogenase (27 kDa), which had previously been described in catfish [9], and 2-phospho-D-glycerate hydro-lyase (50-75 kDa). In addition, we showed the exclusive implication of parvalbumin in the hypersensitivity reaction to sea bass. The observations made above reveal the importance of identifying and characterizing the allergens involved in an allergic reaction in order to establish avoidance measures and indications [9-10].

In conclusion, we present 2 red mullet allergens weighing 27 kDa and 50-75 kDa that were identified, respectively, as glyceraldehyde-3-phosphate dehydrogenase and 2-phospho-D-glycerate hydro-lyase. In this case, cosensitization to parvalbumin and other allergens that are less stable than parvalbumin affects the raw forms. To our knowledge, this is the first time that these proteins have been reported as allergens in red mullet. Our study provides additional information for

the implementation of relevant extracts and components to ensure an accurate diagnosis of fish allergy and reinforces the usefulness of advanced in vitro techniques to unveil difficult allergy cases in which routine tests are not conclusive.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Xepapadaki P, Christopoulou G, Stavroulakis G, Freidl R, Linhart B, Zuidmeer L, et al. Natural History of IgE-Mediated Fish Allergy in Children. J Allergy Clin Immunol Pract. 2021;9(8):3147-56.e5.
2. Ojeda P, Sastre J, Olaguibel JM, Chivato T; investigators participating in the National Survey of the Spanish Society of Allergology and Clinical Immunology Alergológica 2015. Alergológica 2015: A National Survey on Allergic Diseases in the Adult Spanish Population. J Investig Allergol Clin Immunol. 2018;28(3):151-64.
3. Kalic T, Radauer C, Lopata AL, Breiteneder H, Hafner C. Fish Allergy Around the World-Precise Diagnosis to Facilitate Patient Management. Front Allergy. 2021 Oct 13;2:732178. Erratum in: Front Allergy. 2021 Nov 08;2:797456.
4. Kourani E, Corazza F, Michel O, Doyen V. What Do We Know About Fish Allergy at the End of the Decade? J Invest Allergol Clin Immunol. 2019;29(6):414-21.
5. Santos AF, Riggioni C, Agache I, Akdis CA, Akdis M, Alvarez-Perea A, et al. EAACI guidelines on the diagnosis of IgE-mediated food allergy. Allergy. 2023;78(12):3057-76.
6. Dramburg S, Hilger C, Santos AF, de Las Vecillas L, Aalberse RC, Acevedo N, et al. EAACI Molecular Allergology User's Guide 2.0. Pediatr Allergy Immunol. 2023;34 Suppl 28:e13854.
7. Kuehn A, Hilger C, Lehners-Weber C, Codreanu-Morel F, Morisset M, Metz-Favre C, et al. Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. Clin Exp Allergy. 2013;43(7):811-22.
8. Haroun Díaz E, Martín-Pedraza L, Betancor D, Somoza ML, Blanca-López N, Vázquez de la Torre M, et al. Selective Allergy to Whiff (Lepidorhombus whiffiagonis): Identification of Enolase as a New Major Allergen. J Investig Allergol Clin Immunol. 2023;33(1):45-7.
9. Ruethers T, Taki AC, Karnaneedi S, Nie S, Kalic T, Dai D, et al. Expanding the allergen repertoire of salmon and catfish. Allergy. 2021;76(5):1443-53.
10. Villalta D, Scala E, Deleonardi G, Busa M, Rivolta F, Boni E, et al. A comprehensive molecular approach in fish allergy: Usefulness in daily clinical practice. Clin Chim Acta. 2022;533:104-8.

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