Selective Allergy to Conger Fish due to Parvalbumin

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Fish is one of the most frequent causes of food allergy, affecting up to 0.3% of the world's population [1]. Most fish-allergic patients show marked clinically relevant cross-reactivity, while a minority of patients experience selective allergy to specific fish species, with good tolerance to other fish families [2].

We report the case of a 32-year-old woman with mild rhinoconjunctivitis due to pollens and animal dander. In 2017, she developed generalized urticaria, cough, oral pruritus, dysphagia, and abdominal pain immediately after ingestion of a small piece of fideuá, a typical Spanish dish made with noodles, prawns, squid, and fish, which in this case was conger, although hake or snuff are more frequently used. Conger belongs to the subclass Actinopterygii, order Anguilliformes, which also includes eel and moray. Broth made from the head, thorns, and skin of fish is used as water for cooking fideuá. The patient's condition improved hours after symptomatic treatment in the emergency department. She subsequently tolerated pasta and several other types of fish (eg, hake, monkfish, cod, sardine, tuna, salmon, and swordfish).

The allergy work-up included the following (see Supplementary Material):

- Skin prick-tests with commercial extracts and prick-by-prick tests with foods, which yielded positive prick-by-prick results to both raw conger body (12×11 mm) and cooked conger body (10×9 mm).
- Serum specific IgE (kU_A/L) using ImmunoCAP, which yielded positive results to eel (0.81), hake (0.74), rooster (0.5), carp parvalbumin (rCyp c 1) (0.7), and cod parvalbumin (rGad c 1) (0.65) and negative results to cod, salmon, sole, sardine, and anchovy.

Good tolerance to prawns and squid was also confirmed. The patient was diagnosed with anaphylaxis due to conger allergy, and a conger-free diet was recommended.

SDS-PAGE was performed under reducing and nonreducing conditions (Supplementary Material). No relevant differences between both conditions were revealed, suggesting that the proteins involved were mainly monomeric proteins.

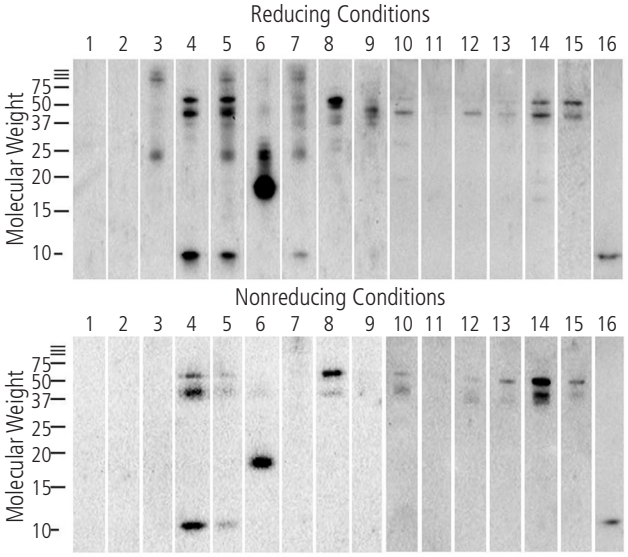

Figure. IgE-immunodetection performed with the patient's serum and the following extracts: Lane 1, Eel; 2, Eel skin; 3, Conger head; 4, Conger body; 5, Conger bone; 6, Conger eye; 7, Conger skin; 8, Salmon; 9, *Anisakis*; 10, Tuna; 11, Cod; 12, Carp; 13, Sole; 14, Hake; 15, Sardine; 16, Cooked conger.

Immunoblotting with the patient's serum and the above-mentioned extracts (Figure) showed that IgE recognized multiple bands, including the following:

1. A 40-50–kDa band, which was detected in raw conger and in all other tested raw fish extracts, but not in cooked conger.
2. A 12-kDa band, which was detected only in raw and cooked conger, but was absent in all other fish tested.
3. A 18-kDa band, which was detected only in the conger eye extract.

The eye of the conger is the part of the head used for making the broth of fideuá. This band was not further studied, because fish eyes are not eaten in Spain and the patient had not experienced problems with broth from other types of fish.

Immunoblotting-inhibition was performed with carp and conger extracts under reducing and nonreducing conditions, and the patient's serum was preincubated with conger extract. As a result, IgE no longer recognized the proteins in the extracts, thus indicating that primary sensitization was probably due to conger. Disappearance of the 40-50–kDa bands suggests that these proteins were similar in both extracts.

Peptide mass fingerprinting was performed with conger extract using spectrometry to characterize the 12-kDa band, since this was thought to have induced the patient's reaction. The band was both conger-specific and thermoresistant. The 4 most relevant peptides were selected after a process of enzymatic digestion, and a specific search for the MASCOT peptide sequence combining MS (proteins) and MSMS (peptides) was performed in NCBI Chordata. The only match found was for an 11–amino acid peptide with the ß-parvalbumin of the fish *Scleropages formosus* in 1 of the 4 peptides (Supplementary Figure). This 11–amino acid peptide has a homology of >80% with many other fish parvalbumins.

Thus, the 12-kDa conger allergen we identified proved to be a ß-parvalbumin.

In terms of gastronomy, conger is one of the 30 main commercial fish species in Europe. Only 3 cases of mild conger allergy have previously been reported, and in all 3 the patients had multiple fish allergies. In addition, the proteins involved were not identified [3,4]. *S formosus* is also known as Malay tongue. It belongs to the order Osteoglossiformes, which is very distant from the order Anguilliformes. To our knowledge, it has never been reported to cause allergic reactions.

ß-Parvalbumins are the main fish allergens and are recognized by 95% of fish-allergic patients [5]. Although ß-parvalbumins are considered to be highly cross-reactive, especially between closely related fish species, isolated clinical allergy to a single fish species has been described for swordfish, tuna/marlin, salmon, sole, tilapia, and pangasius/tilapia [6]. We think this ß-parvalbumin probably behaves as a selective allergen of the Congridae family, because it was not recognized in the other fish extracts tested, including eel extract, and the patient tolerated all other fish species (both cooked and raw). We think that our commonly identified LFLQNFASGAR sequence does not include relevant IgE-binding epitopes and that clinically relevant conger parvalbumin epitopes must be located in different parts of the protein and show no homology with other parvalbumins, thus explaining the lack of cross-reactivity between conger allergens and other allergenic parvalbumins in fish.

Parvalbumins are classified into 2 different families, namely, α and ß parvalbumins. α-Parvalbumins are present in birds, amphibians, cartilaginous fish, mammals, and crocodiles. To date, the only reports of allergy caused by α-parvalbumin involved one patient with allergy to frog leg and another with allergy to crocodile and cartilaginous fish [7,11]. In contrast, ß-parvalbumins are present in bony fish, especially white fish, and are highly allergenic. They have a single 113–amino acid chain, with 2 specific calcium-binding sites. ß-parvalbumins are thermostable proteins with a molecular weight of around 12-14 kDa. In addition, they are resistant to denaturation and enzymatic digestion, which can cause severe reactions [6]. Fish allergenicity depends on the amount of white muscle and processing (canned, cooked, raw) [8].

The 40-50–kDa protein recognized by the patient in the present report is probably enolase or aldolase [9], the second most frequent fish allergens (albeit with doubtful clinical relevance), which are recognized by around 50% of patients. These antigens cannot be responsible for symptoms with cooked conger, since they are thermolabile proteins. Furthermore, they have no clinical relevance in the present case, given that the patient did not have symptoms with other raw fish species. Less frequent fish allergens that have been described include collagen, tropomyosin, aldehyde dehydrogenase, and protamine. In some cases, they seem to be species-specific.

In summary, we report the first case of anaphylaxis due to conger allergy. We also describe the first allergen in conger (ie, a ß-parvalbumin), and a new selective parvalbumin in fish. Interestingly, conger can also behave as a hidden allergen, since it is used to add a fish flavor to typical dishes.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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