Selective allergy to conger fish due to parvalbumin

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Fish allergy is one of the most frequent causes of food allergy, affecting up to 0.3% of the world population [1]. Most fish-allergic patients show high clinically-relevant cross-reactivity, while a minority of patients suffer from selective allergy to certain fish species, with good tolerance to the remaining fish families [2].

We report a 32 year-old woman with mild rhinoconjunctivitis due to pollens and animal dander. In 2017, she developed generalized urticaria, cough, itchy mouth, dysphagia and abdominal pain immediately after the ingestion of a little bit of *fideuá*, a typical Spanish dish made with noodles, prawns, squid and fish, which was conger in this case, although hake or snuff are more used. Conger belongs to subclass *Actinopterygi*, order *Anguilliforme*, which also includes eel and moray and broth of the head, thorns and skin of fish, after removing head and thorns, is used as water for cooking fideua. Once in the Emergency Department, she improved hours after symptomatic treatment. Subsequently, she had tolerated pasta and several fishes (such as hake, monkfish, cod, sardine, tuna, salmon and swordfish).

Allergy study included (see Supplementary file):

- Skin prick-tests (SPT) with commercial extracts and prick-by-prick tests (PP) with foods showed positive PP to both raw (12x11 mm) and cooked conger body (10x9 mm).
- Serum specific IgE (kUA/L) using ImmunoCAP system showed 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; while negative to cod, salmon, sole, sardine and anchovy.

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

SDS-PAGE was performed under reducing and non-reducing conditions (see Supplementary file). Relevant differences between both conditions were not revealed, suggesting that we were dealing with mainly monomeric proteins. Immunoblotting with our patient's serum and the above detailed extracts (Figure 1) showed IgE recognition of multiple bands, including:
1. 40-50 kDa band: detected in raw conger and in all other tested raw fish extracts, but not in cooked conger.
2. 12 kDa band: displayed only in both raw and cooked conger, but absent in all other fish tested.
3. 18 kDa band: showed only in conger's eye extract.

Conger eye is part of the head used for making the broth of the fideua. This band was not further studied because in Spain fish eyes are not eaten, and our patient did not have problems with broth from other fishes.

Immunoblotting-inhibition assay was performed with carp and conger extracts, under reducing and non-reducing conditions, pre-incubating patient's serum with conger extract. As a result, IgE recognition to all proteins disappeared in both extracts, indicating that primary sensitization was probably due to conger. Disappearance of 40-50 kDa bands suggests that these proteins were similar in both extracts.

Peptide mass fingerprinting was performed with conger extract by spectrometry to characterize the 12 kDa band, giving that it was the candidate to have induced patient's reaction, being both conger-specific and thermostable. Four most relevant peptides were selected after a process of enzymatic digestion, and a specific search for peptide sequence MASCOT combined MS (proteins)+MSMS (peptides) in NCBI Chordata was performed. Only a single match of an 11 amino-acid (AA) peptide with the beta-parvalbumin of *Scleropages formosus* fish was obtained in one of the four peptides and it is expressed in Supplementary figure. This 11-amino-acid peptide has an homology higher than 80% with many other fish parvalbumins. Then, our 12 kDa conger allergen turned out to be a beta-parvalbumin.

Gastronomically, conger is one of the 30 main fishing species of commercial interest in Europe. Only 3 patients with mild conger allergy have been previously reported, all of them showed multiple fish allergies and proteins involved were not identified [3,4]. *S. formosus* is a fish known as Malay tongue, belonging to the order Osteoglossiforme, far away from the order Anguilliforme, and to our knowledge never described as a cause of allergic reactions.

Beta-parvalbumins are the main fish allergens, recognized by 95% of fish-allergic patients [5]. Although beta-parvalbumins are considered to be highly cross-reactive, specially between closely related fishes, clinical isolated allergy to single fish species has been described for swordfish, tuna/marlin, salmon, sole, tilapia and pangasus/tilapia [6]. We think this beta-parvalbumin, probably behaves as a selective allergen of the Congridae family because it was not recognized in the other tested fish extracts, including eel extract, and our patient tolerated all other fishes both cooked and raw. We think that our identified common LFLQNFASGAR sequence does not include relevant IgE-binding epitopes, and that clinically-relevant conger-parvalbumin epitopes must be located in other parts of the protein, without homology to other parvalbumins, thus explaining the lack of cross-reactivity of conger with other fish allergenic parvalbumins.

Alpha and beta families of parvalbumins have been described. Alpha-parvalbumins are present in birds, amphibians, cartilaginous fish, mammals and crocodile. So far, only...
one case of allergy to frog legs, and another to crocodile and cartilaginous fishes due to alpha-parvalbumin have been reported [7,11]. In contrast, beta-parvalbumins are present in bone fishes, especially white fishes, and are highly allergenic. They have a single chain of a 113 amino acid, with two specific binding-calcium sites. They are thermostable proteins with a MW around 12-14 kDa, resistant to denaturation and enzymatic digestion, which can cause severe reactions [6]. Fish allergenicity depends on the amount of white muscle, and the type of cooking (canned, cooked, raw) [8].

Our 40-50 kDa protein recognized by our patient could be probably enolases-aldolases (9), the second most frequent fish allergens with doubtful clinical relevance, recognized by around 50% of patients. These antigens could not be responsible for symptoms with cooked conger since they are thermolabile proteins. Furthermore, they have no clinical relevance in our patient, given that she did not have symptoms with other raw fishes. 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, namely a beta-parvalbumin, and a new selective parvalbumin in fishes. Interestingly, conger can further behave as a hidden allergen, since it can be used to add fishy flavor to typical dishes.

CONFLICTS OF INTEREST

All authors declare to have no conflicts of interest.

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LEGENDS TO THE FIGURES: