Selective allergy to conger fish due to parvalbumin

Argiz L[1], Vega F[1], Castillo M[2], Pineda F[2], Blanco C[1,3]

[1]Department of Allergy. Hospital Universitario de La Princesa. Instituto de Investigación Sanitaria Princesa (IP). Madrid. Spain
[2]Application lab. Diater Laboratories. Madrid. Spain
[3]RETIC ARADYAL RD16/0006/0015, Instituto de Salud Carlos III, Madrid, Spain

Corresponding:
Laura Argiz Álvarez
Hospital Universitario La Princesa. Servicio de Alergia
C/ Diego de León 62. CP 28006. Madrid. Spain.
E-mail: largizalvarez@gmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0412

Key words: Conger fish. Selective allergy. Parvalbumin. Fish allergy. Food allergy.

Palabras clave: congrio, parvalbúmina, alergia selectiva, alergia a pescado, alergia alimentaria.

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 Suplemmentary 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:

doi: 10.18176/jiaci.0412

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 thermoresistant. 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.

This manuscript has not been published elsewhere and is not under consideration for publication elsewhere.

FUNDING SOURCES

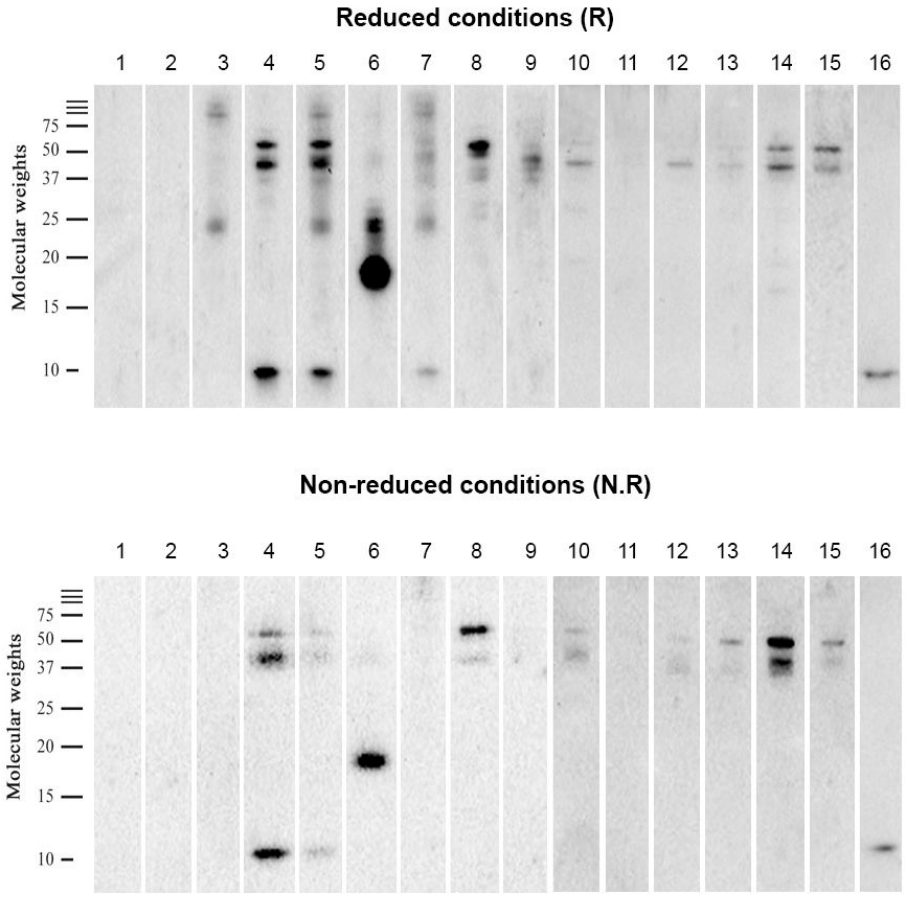
There were no funding sources for this study.

BIBLIOGRAPHY

1. Moonesinghe H, Mackenzie H, Venter C, Kilburn S, Turner P, Weir K, et al. Prevalence of fish and shellfish allergy: A systematic review. Ann Allergy Asthma Immunol. 2016 Sep; 117:264-72.
2. Raith M, Klug C, Sesztak-Greinecker G, Balic N, Focke M, Linhart B et al. Unusual sensitization to parvalbumins from certain fish species. Ann Allergy Asthma Immunol. 2014;113:571-2.
3. Koyama H, Kakami M, Kowamura M, Tokuda R, Kondo Y, Tsuge I et al. Grades of 43 fish species in Japan based on IgE-binding activity. Allergol Internat. 2006;55:311-6.
4. Sugita K, Kabashima K, Nakashima D, Tokura Y. Oral allergy sindrome caused by raw fish in a Japanese sushi bar worker. Contact Dermatitis. 2007;56:369-70.
5. Kourani E, Corazza F, Michel O, Doyen V. What we know about fish allergy by the end of the decade? J Investig Allergol Clin Immunol. 2019 Feb 11:0. doi: 10.18176/jiaci.0381
6. Kuehn A, Swoboda I, Arumugam K, Hilger C, Hentges F. Fish allergens at a glance: variable allergenicity of parvalbumins, the major fish allergens. Front Immunol. 2014;5:179
7. Hilger C, Grigioni F, Thill L, Mertens L, Hentges F. Severe IgE-mediated anaphylaxis following consumption of fried frog legs: definition of alpha-parvalbumin as the allergen in cause. Allergy. 2002;57:1053-8.
8. Sletten G, Van Do T, Lindvik H, Egaas E, Florvaag E. Effects of industrial processing on the immunogenicity of commonly ingested fish species. Int Arch Allergy Immunol. 2010;151:223-36.
9. 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: 811-22.
10. Ballardini N, Nopp A, Hamsten C, Vetander M, Melén E, Nilsson C, et al. Anaphylactic Reactions to Novel Foods: Case Report of a Child With Severe Crocodile Meat Allergy. Pediatrics. 2017 Apr;139(4).

LEGENDS TO THE FIGURES:

Figure 1. IgE-immunodetection performed with our patient's serum, of the following extracts: 1. Anguilla, 2. Eel skin, 3. Conger head, 4. Conger body, 5. Conger eagle 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.



doi: 10.18176/jiaci.0412