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RESEARCH LETTER

WILEY

Identification of myosin light chain protein as a major fish allergen

To the Editor,

The prevalence of food allergy has been increasing worldwide in the last 10 years. Currently, 2%–8% of the population in Western countries suffers from some type of food allergy. In Spain, seafood allergy has increased approximately 30% in the last decade, affecting both children and adults, with 10% affected by fish allergy.[1]

In this study, we have performed an initial screening of the allergens involved in the sensitization of 21 fish allergic patients from the South of Europe, considering: medical record and positive Skin Prick Test (SPT) to one of these five fish extracts (homemade, online repository): cod, hake, tuna, sole and sea bream (Table 1). Western blot was used to recognize the sensitization of these patients and LC/MS was used to characterize the allergen recognized by western blot. For practical reasons, clinical symptoms were recorded and classified into four categories: oral allergy syndrome (OAS), gastrointestinal symptoms (GI), urticaria-angioedema (U/AE) and anaphylaxis (ANA). The study was approved by the Medical Ethical committee of ASISA Dr. Lobatón according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from each patient. Complementary study data can be found at https://zenodo.org/record/6476637#.Yo68HS8lNQI.

Angioedema was the most prevalent symptom, affecting 52% of fish allergy patients, followed by anaphylaxis symptoms (28%). The SPT positive results were: 62% sea bream, 38% sole, 47.61% hake, 38.09% tuna and 38.09% cod.

Only 50% of the patients had a value >0.35 KU/L to sIgE cod using the ImmunoCAP System, and 75% of these patients were parvalbumin sIgE positive. Considering all patients, only 37% were sIgE positive to parvalbumin.

Piruvate kinase (50 KDa), β-enolase (48 KDa), glyceraldehyde dehydrogenase (36 KDa) and parvalbumin (11 KDa) were identified by LC/MS compared with a non-redundant protein database of *Actinopterygii*, using MaxQuant software. These proteins were previously identified as fish allergens[2] (Figure 1A). Also, the fragmentation spectra obtained by the LC/MS analysis in gradient detection mode led three amino acid sequences of the tryptic peptides: VFDKEGNGTVMGAELR, VAYNQIADIMR and EGNGTVMGAELR with a sequence coverage of 13.9896 of myosin light chain of *Pennahia argentata*.

Sensitization profile to a full extract of sea bream were study by SDS-PAGE, IgE-Immunoblotting and LC/MS. Parvalbumin: 11–11.5 KDa (24%–14% of patients respectively), myosin light chain 20 KDa (71%), glyceraldehyde dehydrogenase or tropomyosin: 32–36 KDa (52%–57% respectively), β-enolase: 48 KDa (52%) and pyruvate kinase: 50 KDa (38%) (Figure 1A–C). 41 KDa band recognized by 95% of patients could not be considered because our pool of non-allergic people recognized this band, which was characterized as alpha-actin.

Briefly, we observed 16 profiles of sensitization, being myosin light chain, the most frequent allergen detected in our population (71%) and parvalbumin the less allergen recognized (38%). These results agree with the sIgE analysis, in which only 37% of patients had positive results to Gad c 1. Clinical symptoms seem to vary depending on sensitization IgE profile found it. In that way, patients monosensitized to myosin light chain suffered from symptoms of urticaria, angioedema or anaphylaxis. One patient sensitized to myosin light chain and parvalbumin suffered from symptoms of urticaria and angioedema and one patient was sensitized to both glyceraldehyde dehydrogenase bands associated with oral allergy symptoms. The combination of myosin light chain with other allergen produced different symptoms: urticaria, angioedema or gastrointestinal symptoms (complementary data).

In this study, we have noted that the response to SPT of the different type of fish were different so that would be relationship with the homology of the different allergen depending on the species studied. So, we have studied the homology of myosin light chain in *Sparus aurata*, *Cyprinus carpio*, *Thunnus thynnus* and *Gadus morhua* using the neighbour joining method. Distance was considered when the value was higher than 0.16.[3] We could observe that myosin light chain from *Sparus aurata*, *Cyprinus Carpio* y *Thunnus thynnus* were the nearest to *Gadus morhua* in the phylogenetic tree, although the difference was lower than 0.16. It should be noted that *Solea senegalensis* belonged to other group with respect to *Sparus aurata* (0.478567 units), showing a low similitude between them (25.58%). Also, we studied the similitude between *Sparus aurata* and *Penaeus vannamei*, showing a distance in the sequence of 0.783224 units, which agrees with the similitude in the sequence of peptide 34.23% (ROT75420.1) OR 21,48% (ROT78605.1). Six fish and crustaceous allergic patients were studied by SDS-PAGE with an extract of *Penaeus kerathurus* did not recognized myosin light chain band.

Tomm JM et al. 2013,[4] detected one patient allergic to cod sensitized to myosin light chain in the studied population (8%). In our study, we have identified myosin light chain as an allergen responsible for the sensitization of 71% fish-allergic patients, but it has been identified previously in shrimp.[5] Myosin light chain has been characterized using the homology in the peptide sequence of *Pennahia argentata* which belong to the same Class (Actinopterygii) and Order (Peciformes) as *Sparus aurata*, differencing in the genus classification, Sciaenidae or Sparidae, respectively, with GI number 7678731. *Sparus aurata* is one of the most important fishes in saline and hypersaline aquaculture. It is consumed fresh and steamed, pan-fried, broiled, microwaved, and baked. It is distributed by Eastern Atlantic: British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands; also, in the Mediterranean Sea. Furthermore, it has been found in the Black Sea. In New Zealand it is referred as *Pagrus auratus*.[6]

None of the patients with symptoms to crustacean allergy studied by SDS-PAGE and IgE-recognition in an extract of *Penaeus kerathurus*, showed recognition of myosin light chain of crustaceous (Figures S1–S3, Supplementary Data).

Key messages

- We screened for relevant allergens in 21 fish allergic patients from Southern Europe.
- We identified myosin light chain, glyceraldehyde dehydrogenase and enolase as important fish allergens.
- Accurate diagnosis for these patients was facilitated by including these allergens as well as parvalbumin.

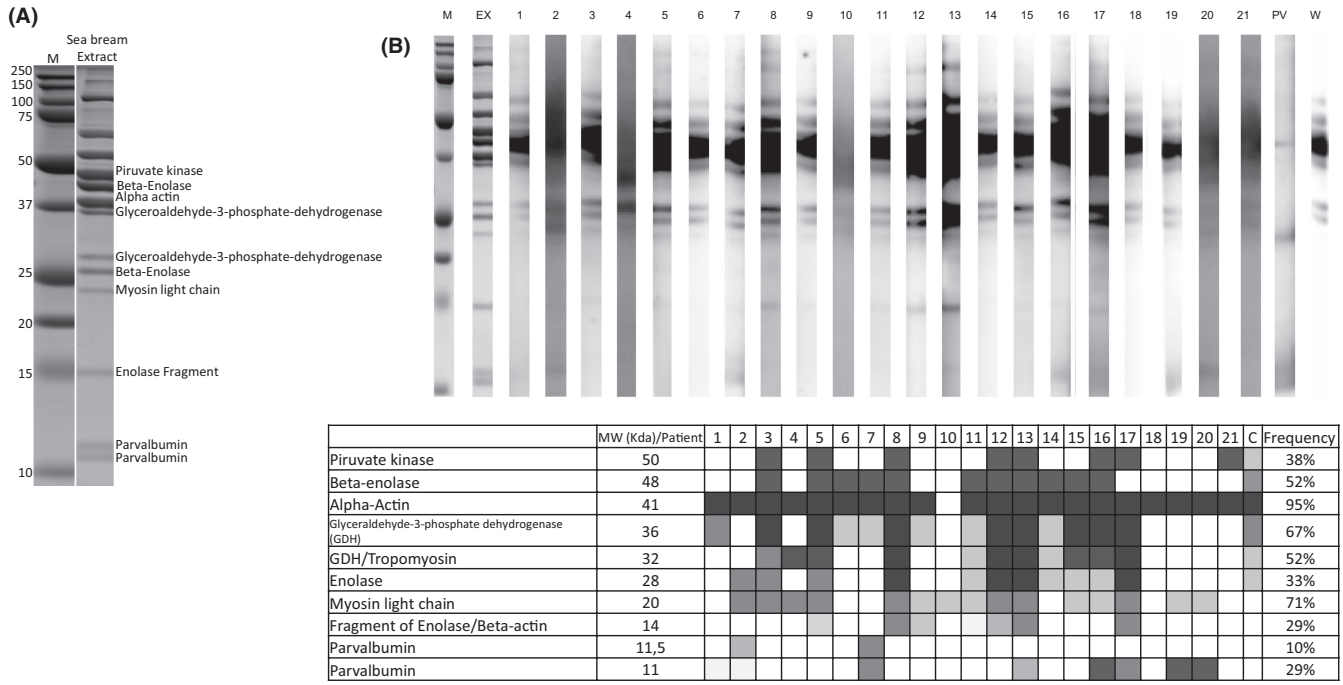
One patient sensitized only to myosin light chain, was studied by TPO showing anaphylaxis reaction.

A high number of studies considered parvalbumin as the main allergen from fish.[7] In this study we show that parvalbumin is only detected by 37% of the allergy according to the SDS-PAGE analysis and this value was very similar to that found in the SPT to cod and Gad c 1 IgE by Immunocap System. *Sparus aurata* was the species of fish to whom patients were most sensitized, close to 75% in our population.

TABLE 1 IgE and prick by SPT of the 21 fish allergic patients

					IgE (kUA/l)			Prick by SPT (mm^2)				
Code	Age	Sex	Symptoms	Fish involved	Total	Tropomyosin	Parvalbumin	Cod	Hake	Tuna	Sole	Sea bream
1	27	F	GI	Hake	24,9	0	0	0	0	0	0	20
2	34	F	OAS	Sea bream	n.d.	n.d.	0	0	7,5	8	8	15
3	15	F	U,AE	Sea bream	572	0	0	0	0	0	0	10
4	35	F	OAS	Sole	82	0	0	0	0	0	20	0
5	42	F	AN	Hake	79,6	0	0	0	6	0	0	12,5
6	32	M	AN	Sea bream*	351	1,96	0	30	35	15	12,5	15
7	9	F	U,AE	Hake	1981	13	16	n.d.	n.d.	n.d.	n.d.	n.d.
8	41	F	AN	Sea bream	n.d.	n.d.	n.d.	0	0	0	0	6
9	13	M	U,AE	Sea bream	248	0	1,64	0	0	10	12,5	0
10	32	M	U,AE	Sea bream	437,9	0	0	0	12	0	0	12
11	52	M	AN	Cod	228	5,03	0	4,5	0	0	0	0
12	37	M	U,AE	Sea bream	387	30	0	6	0	0	7,5	9
13	10	M	U,AE	Salmon	1831	0	0	18	12,5	14	15	20
14	28	F	U,AE	Sea bream	278	0	0	0	8	8	0	16
15	13	M	AN	Hake**	665	0,8	4,6	16	18	0	40	32
16	37	M	AN	Hake	2979	0	0	0	8	0	0	0
17	44	F	GI	Cod	62,24	0	n.d.	4,5	0	12,5	0	12
18	79	M	U,AE	Cod	805	17,6	0	0	0	0	0	0
19	6	M	U,AE	Sea bream	997	100	48	12	14,5	8	17,5	11,5
20	8	M	U,AE	Salmon	1614	n.d.	n.d.	12	8	7,5	n.d.	n.d.
21	12	M	U,AE	Hake	n.d.	0	0	n.d.	n.d.	n.d.	n.d.	n.d.

*TPC with Seabream: systemic reaction (facial erythema, conjunctival hyperaemia and pharyngeal bolus) that motivates doses of systemic steroid and intramuscular adrenaline. **TPC with white fish: negative (Tolerates); CPT, negative, but after 48 h, after eating white fish, he developed pharyngeal itching, upper dysphagia, lip oedema and abdominal pain (ANAPHYLAXIA). Symptom Abreviature: GI (Gastrointestinal), OAS (Oral Allergy Syndrome); U (Urticaria); AE (Angioedema); AN (Anaphylaxis).



	MW (Kda)/Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	C	Frequency
Piruvate kinase	50																							38%
Beta-enolase	48																							52%
Alpha-Actin	41																							95%
Glyceraldehyde-3-phosphate dehydrogenase (GDH)	36																							67%
GDH/Tropomyosin	32																							52%
Enolase	28																							33%
Myosin light chain	20																							71%
Fragment of Enolase/Beta-actin	14																							29%
Parvalbumin	11,5																							10%
Parvalbumin	11																							29%

FIGURE 1 (A) SDS-PAGE protein profile using a full extract of Sea bream (Lane 1). MW: molecular weight (in KDa), (B) Patient IgE immunoblots from SDS-PAGE of *Sparus aurata* extract, revealed with horseradish peroxidase-conjugated goat anti-human-IgE. (C) Allergogram of 21 fish-allergic patients and pool of non-allergic volunteers ($n = 10$)

Myosin light chain is seen in the vast majority of the 21 fish allergic patients studied. Patients with sensitization to parvalbumin and myosin light chain suffered from systemic reactions such as anaphylaxis and urticaria/angioedema (complementary data).

Shibata, Y, et al. 2019, studied seven patients with fish allergy and atopic dermatitis, observing that six of them were sensitized to myosin heavy chain.[8]

Phylogenetic tree between myosin light chain of different fish species showed that *Solea senegalensis* was far from *Sparus aurata*, while the other species aligned closely (*Thunnus thunni*, *Gadus morhua* and *Cyprinus Carpio*). Also, we have observed a large distance between myosin light chain from *Sparus aurata* and *Penaeus vannamei*, observing a distance in the sequence of 0.783224 units. This distance could explain why we did not observe any specific IgE band to myosin light chain in the study by western blot to an extract of *Penaeus kerathurus*.

The main issue in allergic fish studies is the low development of fish-specific allergens for in vitro diagnosis, forcing investigators to do inhibition experiments to confirm the specific allergen in order to give the best advice to patients, making it very difficult to obtain an accurate diagnosis in these patients.[9] Thus, the development of new diagnostic techniques is necessary.

Therefore, myosin light chain, which was recognized by 71% of the allergic patients in our study, may play an important role as a fish allergen. These results will need to be replicated with larger sample sizes to clarify the role of myosin light chain in fish allergy. Thus, it will be interesting to study the amount present of this allergen in different, highly-consumed fish species.

In conclusion, allergens such as myosin light chain, glyceraldehyde dehydrogenase and enolase should be studied in the sensitization of fish-allergic patients in addition to parvalbumin, at least in our area. Investigating for parvalbumin alone did not allow an accurate final diagnose of fish allergy.

AUTHOR CONTRIBUTION

Francisco Moreno Benítez (FMB) is the principal investigator of the study, designed the study and wrote the manuscript. Also, FMB recruitment the patients. Marisa Espinazo Romeu (MER) collected data, analysed results, wrote the manuscript and created the Figure. Antonio Letrán Camacho (ALC) recruitment the patients and collected clinical data. Aurora Jurado Roger (AJR) and Carmen Moreno Aguilar (CMA) wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/record/6476637#.YmHzQy8lNQI, reference number DOI10.5281/zenodo.6476637.

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