

# **Myosin heavy chain, an allergen involved in anaphylaxis to shrimp head**

Saenz de San Pedro B<sup>1</sup>, López Guerrero A<sup>1</sup>, Navarrete del Pino MA<sup>1</sup>, Alcántara Villar M<sup>1</sup>, Álvarez F<sup>2</sup>, López-Matas MA<sup>2</sup>, Carnés J<sup>2</sup>

<sup>1</sup>Allergology Unit. Hospital Universitario Jaén, Spain.

<sup>2</sup>R&D Unit, LETI Pharma S.L.U., Tres Cantos, Madrid, Spain.

## **Corresponding author:**

Jerónimo Carnés, PhD

Research & Development

Laboratorios LETI, S.L.U.

Calle del Sol nº 5

28760 Tres Cantos

E-mail: jcarnes@leti.com

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**Palabras clave:** Alergia alimentaria, Alergia a mariscos, Cabezas de gambas, Alérgeno, Cadena pesada de miosina.

Seafood is one of the most prevalent food allergenic sources and is becoming a leading global cause of anaphylaxis [1-3]. Nine allergens have been reported in shrimp to date ([www.allergen.org](http://www.allergen.org)). Allergy to shrimps is generally diagnosed by preparations containing shrimp bodies. This could account for misdiagnosing or even a false negative result for patients consuming shrimp heads. In this study, we investigated a patient with allergic symptoms after shrimp heads consumption and we identified a myosin heavy chain (MHC), as the allergen responsible.

The patient was a 30-year-old male with multiple episodes of pruritic generalized rash, dyspnoea and dysphagia, approximately seven to eight hours after the ingestion of crustaceans. In the last episode, after the ingestion of shrimp, the patient required emergency care with a good response to intravenous medication and notified one sphincter relaxation episode with loss of consciousness. No cofactors were associated with the reaction. He occasionally tolerates crustaceans and has good tolerance to molluscs and fish.

Skin prick test was positive to Anisakis (Roxall Group, Bilbao, Spain) (5 mm  $\emptyset$ ) and prick-by-prick was positive to shrimp heads (8 mm  $\emptyset$ ) and crayfish heads (5mm  $\emptyset$ ) and negative

to shrimp and crayfish bodies. Testing of heads and bodies was performed separately because the patient usually sucks the crustacean heads. Total IgE was 113 kU/L and specific IgE 11 kU/L to Anisakis, 0.48 kU/L to shrimp and negative to tropomyosin and mites (*Dermatophagoides pteronyssinus* and *Lepidoglyphus destructor*) (ImmunoCAP™, Thermo Fisher Scientific, Uppsala, Sweden).

Shrimps (*Penaeus sp.*) were purchased at a local market and used to prepare heads and bodies extracts. The content of the heads was removed with a spatula and the bodies were peeled. Afterwards, both were boiled for 10 minutes in PBS 0.01 M, NaCl 0.15 M, homogenized and kept under agitation at 4 °C during 4 hours. Finally, heads and bodies extracts were centrifuged, dialyzed, sterile filtered and lyophilized. The protein content per mg of lyophilized extract was analyzed by the Bradford method (Thermo Scientific, Rockford, IL, USA) and the results were of 63.1 µg and 576.7 µg in shrimps heads and shrimps bodies, respectively.

SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) technique was used to analyze the protein profile of both extracts. Twenty micrograms of protein of each extract were loaded in the gel and run under reducing conditions. The protein molecular weights were distributed in 10–40 kDa in heads and in 10–150 kDa in bodies (Figure 1B).

The protein bands recognized by the IgE from the patient serum were determined by immunoblotting. Proteins separated by SDS-PAGE were transferred to a Trans-Blot® Turbo™ PVDF membrane (Bio-Rad, Hercules, CA, USA) and incubated overnight with the patient serum diluted 1/2. HRP-conjugated mouse anti-human IgE (Southern Biotech, Birmingham, AL, USA) was used as secondary antibody and ECL™ Prime Western Blotting Detection (Amersham, Little Chalfont, Buckinghamshire, UK) was used to detect the

allergenic proteins by chemiluminiscence. A single band at approximately 40 kDa was detected in shrimp heads and none in shrimp bodies (Figure 1D). Cross-reactivity between shrimp bodies and the protein found in shrimp heads was discarded by immunoblot inhibition (data not shown).

Additionally, a two-dimensional electrophoresis (2D) was employed to investigate the protein profile in more detail. Shrimp heads extract was washed using ReadyPrep 2D Cleanup Kit (BioRad) and proteins were separated according to their isoelectric point in ReadyStrip IPG (BioRad) in a pH range of 3–10. After the first dimension, the proteins were separated in the second dimension according to their molecular weight (MW). Gel was stained with Oriole Fluorescent Gel Stain (BioRad). A second 2D gel run in parallel was transferred to a membrane as previously explained to obtain the 2D allergenic profile.

The protein spots were distributed throughout the entire pH range (Figure 1A). Three different spots were recognized by the patient serum corresponding to isoforms of the 40 kDa protein (Figure 1C). These same spots were cut from the 2D SDS (Figure 1A, red arrows), sequenced by LC-MS/MS (liquid chromatography tandem mass spectrometry) and the obtained peptides were searched in the NCBI-Crustacea database for their identification. A total of fifteen peptides corresponded to a MHC (accession number XP\_027224354.1 [www.ncbi.nlm.nih.gov/protein](http://www.ncbi.nlm.nih.gov/protein)), with a sequence coverage of 57%.

Myosin functions during muscle contraction together with actin. However, it also has other functions such as cytokinesis, cell organization, intracellular transport of organelle or other particles and signal transduction, so its distribution is ubiquitous in all eukaryotic cells throughout all parts of the body [4].

The MHC identified in this study is a thermostable allergen that belongs to the myosin type-2 sub-family. The structure of these proteins consists of two heavy chains with MW

between 171 and 244 kDa which, in turn, are constituted by three subunits of 25, 50 and 20 kDa joined by peptide loops [5]. Each MHC is associated with two myosin light chains (MLC) [6]. In *Litopenaeus vannamei* unconventional myosin genes with high diversity have been described, the authors proposed that alternative splicing could produce myosin variants with different functions [4]. This could be the reason that we only found the allergenic protein MHC in the heads and not in the shrimp bodies were the muscle is the main component. Additionally, the MHC found has a MW of 40 kDa, which is lower than other MHCs.

The allergens Lit v 3 [7] and Pen m 3 are MLC thermoresistant proteins [8]. In addition, MHC has been reported as an allergen from the muscle of banana shrimp (*Fenneropenaeus merguensis*) [9]. None have been reported as exclusive heads allergens.

In conclusion, we described a patient with food allergy caused by the ingestion of shrimp heads. The allergen involved was identified as a MHC. This is the first time a protein from this family has been reported as a shrimp heads allergen. More patients allergic to shrimp heads should be studied to confirm the importance of MHC as an allergen.

### **Conflicts of interest**

B. Saenz de San Pedro has received research support and/or speaker/consultancy fees from Takeda, Novartis, ALK-Abello, Allergy Therapeutics, Merck-Allergopharma, Meda and GSK. She has also received funding to attend conferences/educational events from LETI Pharma, CSL Behring, Takeda, ALK-Abello, Novartis, Astra Zeneca and Roxall. She has been a clinical trial/registry investigator for LETI Pharma, Takeda, ALK-Abello, Allergy Therapeutics, Merck-Allergopharma, Roxall and Stallergens.

F. Álvarez, M. A. López-Matas and J. Carnés are employees of LETI Pharma.

A. López Guerrero has received funding to attend conferences/educational events from LETI Pharma, Immunotek, ALK-Abello and Novartis.

M.A. Navarrete del Pino has received speaker/consultancy fees from Immunotek and GSK. She has also received funding to attend conferences/educational events from LETI Pharma, GSK, Immunotek and ALK-Abello.

M. Alcántara Villar has received speaker/consultancy fees from LETI Pharma, ALK-Abello, GSK, Astra Zeneca, Roxall and Immunotek. He received payment for expert testimony from Allergy Therapeutics. He has also received funding to attend conferences/educational events from GSK.

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## Figure legend

**Figure.** Protein and allergenic profile of the shrimp extracts. A- 2D electrophoresis of shrimp heads. Spots identified by LC/MS-MS are pointed with arrows. B- SDS-PAGE of shrimp heads and bodies. C- 2D immunoblot. D- Immunoblot of shrimp heads and bodies. In both immunoblots the patient serum was diluted ½.

