

# Serological proteome analysis identifies crustacean myosin heavy chain type 1 protein and house dust mite Der p 14 as cross-reacting allergens

Antonio Conti<sup>1,A–F</sup>, Noor Alqassir<sup>1,B,E,F</sup>, Daniela Breda<sup>2,B,C,F</sup>, Alan Zanardi<sup>1,B,C,F</sup>, \*Massimo Alessio<sup>1,A–F</sup>, \*Samuele E. Burastero<sup>2,A–F</sup>

<sup>1</sup> Proteome Biochemistry, COSR-Centre for Omics Sciences, IRCCS-San Raffaele Hospital, San Raffaele Scientific Institute, Milan, Italy

<sup>2</sup> Laboratory of Cellular and Molecular Allergology, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

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## Address for correspondence

Samuele E. Burastero  
E-mail: burastero.samuele@hsr.it

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\*Massimo Alessio and Samuele E. Burastero contributed equally to this work.

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## Abstract

**Background.** Allergies to house dust mite (HDM) and to crustaceans are clinically and pathogenically linked. Several homologous allergenic proteins have been identified, among which tropomyosin is the prototype, expressing epitopes endowed with variable levels of immunoglobulin E (IgE) cross-reactivity. Component-resolved diagnosis (CRD) does not allow a thorough characterization of all relevant IgE reactivities to these allergen sources.

**Objectives.** We studied 1 patient allergic to shrimp with positive skin prick test to HDM and negative scores for IgE to HDM allergen components routinely used in CRD (group 1 and 2 allergens, Der p 23 and tropomyosin).

**Materials and methods.** In order to identify the allergen(s) involved in IgE reactivity, we used serological proteome analysis (SERPA), which utilizes two-dimensional gel electrophoresis (2DE), immunoblotting and mass spectrometry (MS). The identified allergenic proteins were tested with sera from 20 crustacean-allergic patients and 19 grass-allergic patients serving as controls.

**Results.** Der p 14 and myosin heavy chain type 1 (MHC1) were identified as the components recognized by patient's IgE in the proteome of *Dermatophagoides pteronyssinus* and *Penaeus monodon*, respectively. The MHC1 protein shows about 30% sequence identity with Der p 14 in specific domains, and cross-reactivity against epitopes shared by the 2 proteins was demonstrated by reduced reactivity to shrimp extract following pre-incubation with Der p 14. Serum IgE from 5 out of 20 patients allergic to crustaceans reacted with MHC1, compared to none among 19 controls ( $p < 0.05$ ).

**Conclusions.** We identified MHC1 as a relevant allergic component in the proteome of *Penaeus monodon*, the prototypic allergen source used in diagnosis of allergy to crustaceans. Our data demonstrate MHC1 cross-reactivity between MHC1 and Der p 14 from *Dermatophagoides pteronyssinus*.

**Key words:** case report, *Dermatophagoides*, proteomics, allergens, immunoglobulin E

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## Background

Diagnosis of food and inhalant allergies requires the combination of clinical history, physical examination and specific in vitro and in vivo diagnostic tests aimed to identify reactivity of immunoglobulin E (IgE) to the allergen(s) of interest. When used to identify reactivity to individual allergenic components (instead of reactivity to whole allergen extracts), these tests allow component-resolved diagnostics (CRD). Currently, allergens used for CRD are either obtained as purified natural extracts or as recombinant proteins. Commercial availability of individual allergenic components limits the diagnostic opportunities for successful determination of IgE. In fact, patients with verified allergy to a certain allergenic source score negative at CRD analysis when the allergenic component responsible for their IgE reactivity is not included in the molecular allergology-based assays. In such cases, serological proteome analysis (SERPA) combined with protein identification through mass spectrometry (MS) can identify the allergenic protein responsible for IgE reactivity.<sup>1,2</sup>

Here, we report the identification of 1 patient with shrimp allergy and positive skin prick test to house dust mite (HDM) whose serum scored negative for IgE to all HDM allergens routinely used in CRD. Simultaneous IgE reactions to mites and crustaceans is a relatively common occurrence<sup>3</sup> mostly explained by IgE reactivity to tropomyosin.<sup>4</sup> However, several other allergens have been found to be cross-reactive, e.g., arginine kinase, myosin light chain, hemocyanin, and paramyosin.<sup>5</sup> We report the identification of a new mite-shrimp cross-reactive allergen component.

## Objectives

This study aimed to characterize using SERPA the allergen component(s) responsible for IgE reactivity to HDM in 1 patient sensitized to shrimp and mites whose IgE scored negative for commercially available *Dermatophagoides* allergen components. The frequency of this reactivity in a group of allergic patients was also evaluated as part of the preliminarily characterization of the clinical relevance of the allergen responsible for the observed cross-reactivity.

Procedures were performed in accordance with the ethical standards of the San Raffaele Ethics Committee (approval ID BIOL-IMMUNO-ALLERGO, date of approval after revision June 12, 2019) and with the Helsinki Declaration of 1975, as revised in 2000 (project ID: BIOL-IMMUNO-ALLERGO, San Raffaele Scientific Institute, Milan, Italy, revised on June 12, 2019).

## Materials and methods

A graphical presentation of the study is shown in Fig. 1.

## Testing of IgE reactivity

Patients reporting to the allergy clinic of the San Raffaele Scientific Institute (Milan, Italy) are routinely tested using skin prick test with a panel of regionally relevant allergens (conf. Supplementary Materials and Methods). Testing and interpretation of results follows European Academy and Allergy and Clinical Immunology (EAACI) guidelines.<sup>6</sup>

Evaluation of serum IgE reactivity to HDM components was performed in patients scoring positive for IgE to HDM extract, as part of the routine evaluation of patients reporting to our allergy clinic. Patient PT 0321 (a 49-year-old female) had a clinical history of food allergy (generalized urticaria and angioedema) reproducibly correlated with meals containing crustacean-based ingredients. Prick test and IgE to *Dermatophagoides pteronyssinus* scored positive, but she did not report any symptoms compatible with respiratory allergies and respiratory or contact reactions following overt exposure to house dust.

Type of assays used for IgE specific determination are reported in Supplementary Materials and Methods. Frequency of serum IgE reactivity to myosin heavy chain type 1 (MCH1) was studied using western blot in 20 patients allergic to crustacean and in a cohort of 19 patients allergic to grass who served as atopic, non-shrimp allergic controls. Patients allergic to shrimps reported singularly or in various combinations the following symptoms: oral allergy (n = 9), contact urticaria (n = 7), urticaria/angioedema (n = 16), asthma (n = 3), and anaphylaxis (n = 7). Demographic characteristics and profiles of IgE reactivities of patients are shown in Table 1. Two-dimensional electrophoresis (2DE) was performed as described in a study by Conti et al.<sup>2</sup> Details of binding competition assays are reported in Supplementary Materials and Methods.

## Mass spectrometry

For protein identification with MS analysis, preparative gels were stained with colloidal blue Coomassie or MS-compatible silver staining, images were acquired and spots of interest were excised from gels, reduced, alkylated, and in-gel digested with bovine trypsin, as described by Conti et al.<sup>2</sup>

## Statistical analyses

Patients' characteristics were reported cumulatively as median (interquartile range (IQR)) for continuous variables or proportions (percentage) for categorical variables. Differences between proportions were tested with two-tailed Fisher's exact test. Values of  $p < 0.05$  were considered statistically significant.

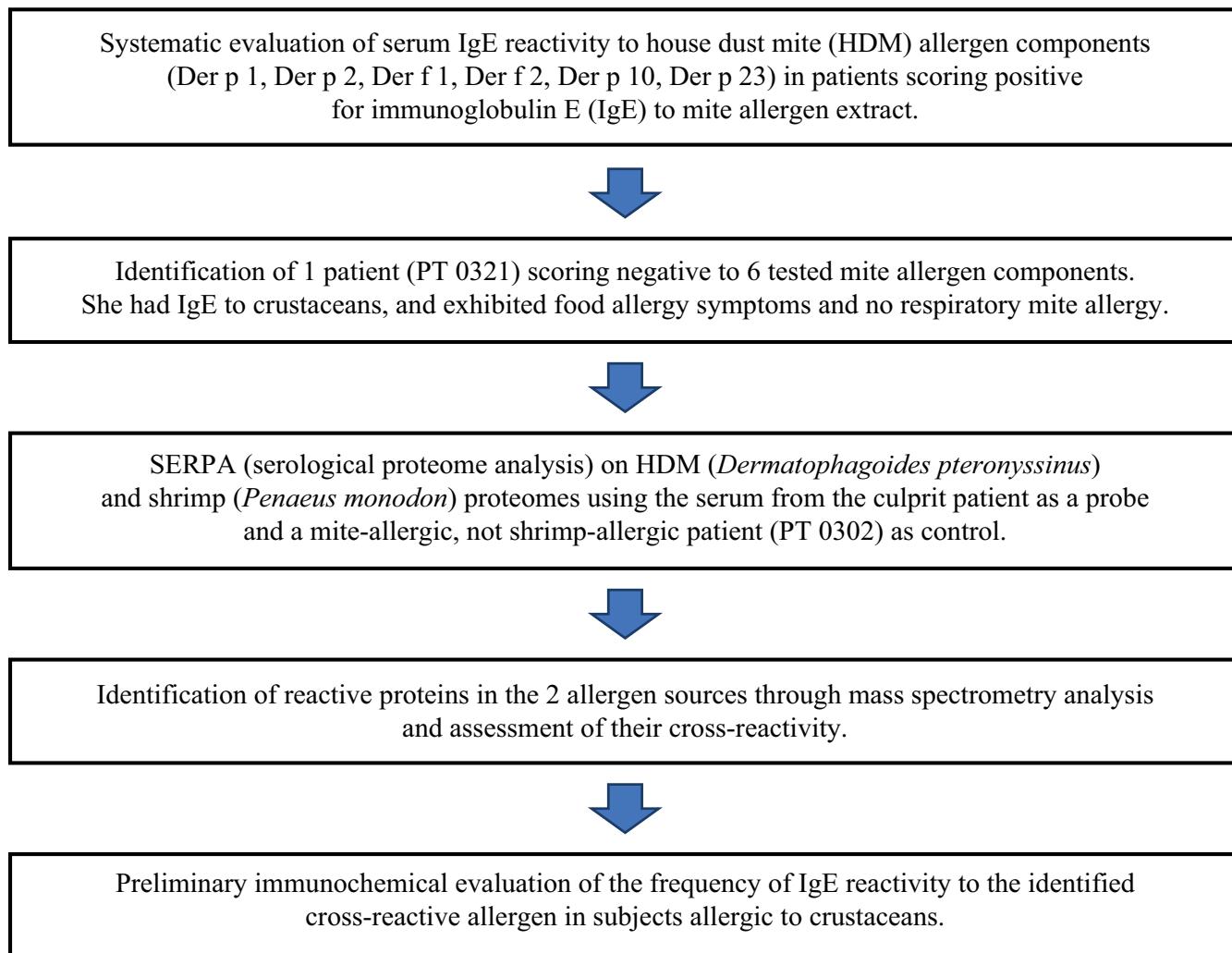


Fig. 1. Graphical presentation of the study

## Results

The skin prick test of patient PT 0321 yielded positive results for IgE to HDM allergens *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*, and negative results for all other geographically relevant allergens that were tested (Fagales, pellitory, ragweed, molds, and animal dander). Patient PT 0321's total IgE level was 44 ng/mL (normal value <100 ng/mL), and specific IgE determination with available routine diagnostic tests yielded negative results for reactivity against all HDM allergens (group 1 and 2 allergens, Der p 23, Der p 23 and for mite tropomyosin, i.e., Der p 10). Specific IgE reactivity to other crustaceans (shrimp, lobster and crab extracts) confirmed the results of prick test. Demographic data and IgE reactivity data from all patients are listed in Table 1.

### Identification of IgE reactive allergen(s) in HDM and crustacean proteomes

The SERPA analysis of patient PT 0321's serum reactivity to HDM protein extract resulted in the identification

of Der p 14 as the major IgE-binding allergen (58 kDa, isoelectric point 5.15 pH; Fig. 2). The SERPA analysis of serum reactivity to shrimp allergen extract allowed for the identification of a 220–240 kDa and 5.44 pH isoelectric point spot compatible with MHC1 (Supplementary Fig. 1). The MS profiles in HDM and shrimp extracts are detailed in Supplementary Tables 1 and 2, respectively. Peptides belonging to Der p 14 allergen identified with MS are shown in Supplementary Fig. 2.

### Cross-reactivity between MHC1 and Der p 14

In silico investigation for potential cross reactivity between Der p 14 and MHC1 based on the comparison of the amino acid sequence of the primary structure of the proteins showed some sequence identity in the range of 27–41% in specific domains. For blast protein alignment, amino acid sequence from UniprotKB (<https://www.uniprot.org>) were Q8N0N0 and K4Q4N8 for Der p 14 and MHC1, respectively. Sequence match analysis was conducted using NIH BLAST website (<https://blast.ncbi.nlm.nih.gov>).

Table 1. Demographic features and serological immunoglobulin E (IgE) reactivity of allergic patients tested for house dust mite (HDM), grass and crustacean allergens

Patient	Sex	Age [years]	Total IgE [ng/mL]	House dust mite						Shrimp						
				<i>D. pteronyssinus</i>			<i>D. farinae</i>			<i>Phleum pratense</i>			<i>Penaeus monodon</i>			
				extract [AU/mL]	Der p 1 [AU/mL]	Der p 2 [AU/mL]	extract [AU/mL]	Der f 1 [AU/mL]	Der f 2 [AU/mL]	extract [AU/mL]	Phl p 1 [AU/mL]	Phl p 5 [AU/mL]	extract [AU/mL]	Pen m 1 [AU/mL]	Pen m 2 [AU/mL]	Pen m 4 [AU/mL]
0321	f	49.0	44.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.2	0.0	0.0
0302	f	45.0	63.0	nd	5.8	4.0	nd	4.0	11.9	0.0	0.3	0.0	0.0	0.0	0.0	0.0
grass (n = 19)	10f	30.1	159.9	0.0	0.0	0.0	0.0	0.0	0.0	23.7	22.4	14.3	0.0	0.0	0.0	0.0
lower IQR	-	22.0	105.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	4.5	4.2	0.0	0.0	0.0	0.0
upper IQR	-	38.0	237.5	0.0	0.0	0.0	0.0	0.0	0.0	20.4	35.1	23.8	0.0	0.0	0.0	0.0
shrimp (n = 20)	5f	28.6	145.9	0.0	0.0	0.0	0.0	0.0	0.0	14.2	19.3	10.1	0.0	0.0	0.0	0.0
lower IQR	-	61	44.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.2	0.7	0.0
upper IQR	-	87.5	280.0	10.1	1.6	1.32	4.3	1.3	9.8	26.5	45.1	18.1	18.6	20.5	0.0	0.0

Der p 1 and Der p 2 – *Dermatophagoides pteronyssinus* allergen p 1 and p 2; Der f 1 and Der f 2 – *Dermatophagoides farinae* allergen f 1 and f 2; Phl p 1 and Phl p 5 – *Phleum pratense* allergen p 1 and p 5; Pen m 1, Pen m 2 and Pen m 4 – *Penaeus monodon* allergen m 1, m 2 and m 4; nd – not determined; grass – grass-allergic patients; shrimp – shrimp-allergic patients; AU – arbitrary units; IQR – interquartile range.

nih.gov/Blast.cgi). Results are provided in Supplementary Fig. 3.

Binding competition with Der p 14 demonstrated the substantial reduction of IgE signal binding to MCH1 (~40% reduction at 0.38 µg MCH1 and ~55% reduction at 0.76 µg MCH1, compared to non-preincubated serum). Data were generated in replicates due to Der p 14 reagent limitation. No reduction was observed in IgE signal at 0.38 µg and ~10% reduction at 0.76 µg with Der f 2 used as a control mite allergen (Supplementary Fig. 4).

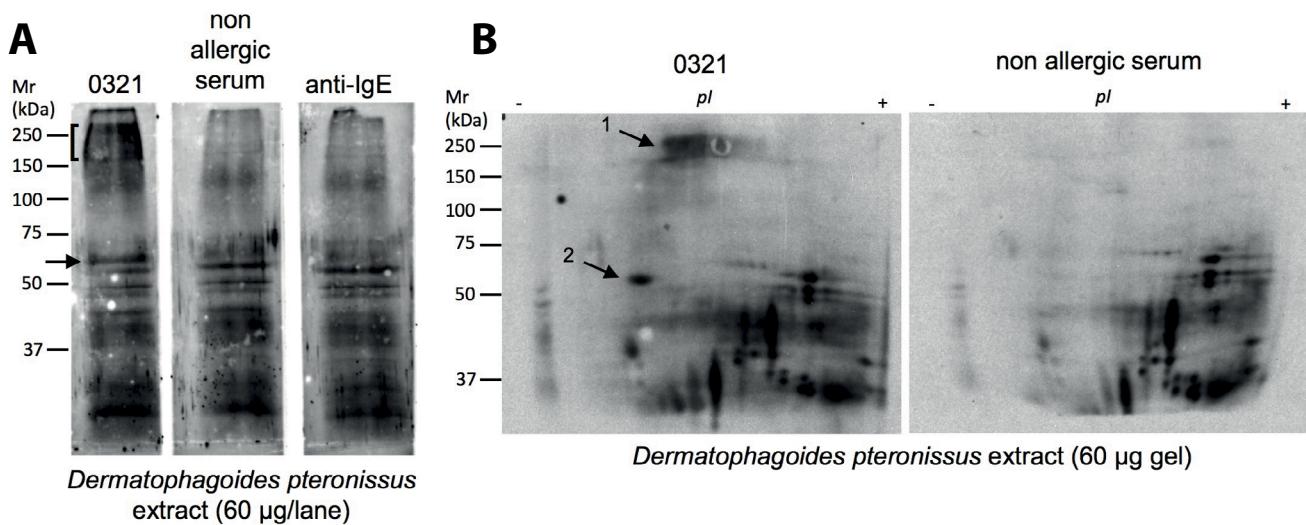
## Evaluation of IgE reactivity to MHC1 protein in a cohort of patients allergic to crustaceans

Following the identification of the spot compatible with MHC1 by means of SERPA analysis based on PT 0321 serum, we made a preliminary estimation of the frequency of IgE reactivity to this allergen in shrimp-allergic patients with immunoblotting. To this aim, we used sera from 20 shrimp-allergic patients who reported to the outpatient clinic of the San Raffaele Scientific Institute. A positive MHC1 immunoblotting signal was detected in 5 out of the 20 patients of this cohort, and 15 of them scored negative. Notably, among these 5 MHC1-positive patients, 3 (including PT 0321) had coexisting sensitization to HDM. A group of 19 subjects allergic to grass served as controls, being representative of an atopic population with a different sensitization profile. Among these 19 subjects, sera scored positive for this marker 0 times and negative 19 times, respectively ( $p = 0.047$ , two-tailed Fisher's exact test).

## Discussion

This paper reports the identification of MHC1 as a relevant allergic component in the proteome of *Penaeus monodon*, which is not presently available in the diagnostic armamentarium of CRD. We show IgE reactivity compatible with MHC1 in 5 out of 20 of patients allergic to crustaceans. We also demonstrate cross-reactivity between MHC1 and Der p 14 from the *Dermatophagoides* proteome.

Our results confirm and expand previous studies reporting that tropomyosin, the originally described pan-allergen of invertebrates, is just one among several highly cross-reactive allergens between crustaceans and mites.<sup>5,7</sup> These findings are in agreement with already reported observations that HDM was able to inhibit shrimp IgE reactivity, or vice versa, in most shrimp-allergic patients.<sup>8</sup> In patients with this sensitization pattern, the primary sensitization may occur via the respiratory or the gastrointestinal tract, likely depending on the characteristics of allergen exposure (frequency, bioavailability, amount, etc.).



**Fig. 2.** Reactivity of the serum of patient PT 0321 to *Dermatophagoides pteronissinus* extract. A. Allergen extract underwent sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and different lanes were probed using western blot for (i) PT 0321 serum, (ii) non-allergic patient serum and (iii) anti-human IgE secondary antibody alone as control; B. Sample extract subject to two-dimensional gel electrophoresis (2DE), and probed using western blot for (i) patients PT 0321's and (ii) non-allergic patient serum. Square bracket and arrows indicate the band or protein spots recognized by patient's 0321 IgE only. Spot 1 and 2 were processed with mass spectrometry (MS) analysis for protein identification

To the best of our knowledge, this is the first time that MCH1 is reported as a mite-shrimp cross-reactive allergen. Although MHC1 from banana shrimp (*Fenneropenaeus merguiensis*) was previously reported as an IgE binding protein, it was not formally classified as a new allergen according to World Health Organization/International Union of Immunology Societies (WHO/IUIS) Allergen Nomenclature subcommittee,<sup>9</sup> and no data on cross-reactivity with other allergens were presented.<sup>10</sup> Shrimp allergy represents an example of coexistence of food and inhalant allergy, as it is the case of allergies triggered by allergen components of the protein families PR-10<sup>11</sup> and LTP.<sup>12</sup> Further work, including cloning of MHC1 or its subunit, is needed to formally characterize this protein as a new allergen.

## Limitations

The MCH1 protein was not cloned, either as a whole molecule or as IgE binding subunit(s). The availability of cloned proteins will allow to characterize MHC1 as a new candidate allergen according to the WHO/IUIS, and to study IgE reactivity in a representative population of patients allergic to shrimp.

## Conclusions

This paper reports the identification of MHC1 as a relevant allergic component in the proteome of *Penaeus monodon*. Our data highlight MHC1 relevance beyond allergy to crustaceans, as we have found cross-reactivity between MHC1 and Der p 14 from *Dermatophagoides*.

## Supplementary Materials and Methods

Detailed description of assays used for IgE specific determination and for binding competition assays are available at doi:10.17632/jbjkntjhkn.1. The package consists of the following files:

Supplementary Table 1. Protein identification using MS in HDM extract.

Supplementary Table 2. Protein identification using MS in shrimp extract.

Supplementary Fig. 1. Identification of IgE reactive allergen(s) from crustacean proteome.

Supplementary Fig. 2. Peptides belonging to Der p 14 allergen identified with MS.

Supplementary Fig. 3. Blast protein sequence alignment between Der p 14 and MCH1.

Supplementary Fig. 4. Competition reactivity test against shrimp extract.

## ORCID iDs

Antonio Conti <https://orcid.org/0000-0001-9150-5271>

Daniela Breda <https://orcid.org/0000-0003-2807-0089>

Alan Zanardi <https://orcid.org/0000-0002-8555-279X>

Massimo Alessio <https://orcid.org/0000-0002-4133-3472>

Samuele E. Burastero <https://orcid.org/0000-0001-7302-6381>

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