

## Two new allergens in a watermelon (*Citrullus lanatus*) allergy episode

Carpio-Escalona LV<sup>1,3\*</sup>, Fernández-Lozano C<sup>2,3,\*</sup>, Peracho L<sup>4,5\*</sup>, Martínez-Alonso E<sup>4,5</sup>, Rita CG<sup>6</sup>, Martínez-Botas J<sup>2,7</sup>, Alcázar A<sup>4,5,\*\*</sup>, de la Hoz Caballer B<sup>1,8,\*\*</sup>

<sup>1</sup>Allergy Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

<sup>2</sup>Biochemistry-Research Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

<sup>3</sup>University of Alcalá, Madrid, Spain

<sup>4</sup>Department of Research, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

<sup>5</sup>Proteomics Unit, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

<sup>6</sup>Immunology Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

<sup>7</sup>CIBER of Obesity and Nutrition Pathophysiology (CIBEROBN), Spain

<sup>8</sup>Spanish Thematic Network and Co-operative Research Centres, ARADyAL, Spain

\*These authors have equally contributed and should be considered as “first authors”.

\*\*Both authors have equally contributed and should be considered as “senior authors”.

### **Corresponding Author:**

**Laura Carpio-Escalona**

Allergy Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

Carretera de Colmenar Viejo. 28034 Madrid.

E-mail: [lauvce@gmail.com](mailto:lauvce@gmail.com)

### **Post-Publication Corresponding Authors:**

**Alberto Alcázar**

Department of Research, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

Carretera de Colmenar Viejo. 28034 Madrid

E-mail: [alberto.alcazar@hrc.es](mailto:alberto.alcazar@hrc.es)

**Belén de la Hoz Caballer**

Allergy Department, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain

Carretera de Colmenar Viejo. 28034 Madrid

E-mail: [bhozcaballer@gmail.com](mailto:bhozcaballer@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.0804

**Key words:** Alpha-galactosidase. BiP. Cucurbitaceae family. GPR78. Watermelon.

**Palabras clave:** Alergia a cucurbitáceas. Alfa-galactosidasa. Sandía.

Watermelon fruit (*Citrullus lanatus*) belongs to the Cucurbitaceae family together with melon (*Cucumis melo*), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*) and other species. Cross-reactivity allergy among them is widely known. Few reported cases of allergic reactions to watermelon are described as oral allergy syndrome as its main clinical manifestation with lower rates of systemic reactions [1]. In 2009, Pastor et al. reported malate dehydrogenase (36 kDa), triosephosphate isomerase (28 kDa), and profilin (13 kDa) as the three major allergens in watermelon, with frequencies of sensitization of 96, 78 and 56 %, respectively [2]. The most recent case reported describes cucumisin (55 kDa) and phloem lectin Lec 17-1 (18 kDa) showing cross-reactivity with melon [3].

We present a 54-year-old male, with grass pollen allergic rhinoconjunctivitis, who develops dysphonia, oropharyngeal and palmoplantar pruritus and genital swelling without other symptoms, about three hours after the ingestion of a piece of watermelon and few minutes after the ingestion of dates (*Phoenix dactylifera*). The patient was recuperated after dexchlorfeniramine and methylprednisolone administration. In the following two weeks, the patient presented the same symptoms in two mild episodes fifteen minutes after repeated watermelon consumption. Subsequently, he tolerated correctly tree nuts, other fruits including melon and cucurbits.

Skin prick test (SPT) were performed with commercial extracts of watermelon, melon, *Phleum pratense*, *Platanus acerifolia*, palm pollen profilin (Pho d2), peach lipid transfer protein (LTP), dates and tree nuts including cashew, being positive for *Phleum pratense*, *Platanus acerifolia*, Pho d2, watermelon and melon. Also, prick by prick with

watermelon and melon pulp were performed with positive results, as well as skin test with proteins extract from pulp and the inner part of the rind of both fruits.

The total serum of IgE was 29 kU/L, with negative results for watermelon and melon specific IgE (0.04 kU/L and 0.06 kU/L, respectively); with positive results for *Phl p1+* *Phl p5b* (3.98 kU/L) and *Phl p12* specific IgE (0.44 kU/L). All determined with UniCap System; Phadia, Uppsala, Sweden; not available for *Pho d2* in our case. A controlled oral challenge test with dates was performed with negative results.

Protein extracts from watermelon and melon were prepared by homogenization in 20 % w/v phosphate-buffered saline (PBS). The homogenates were centrifuged at 17,700 g for 30 minutes, dialyzed in deionized water (3.5 kDa molecular weight cut-off) and sterilized by filtration (0.22  $\mu$ m filter pore diameter). The allergenic potential of watermelon and melon extracts was confirmed by slot blot to preserve allergen integrity (Figure 1A).

A basophil activation test (BAT) was performed with watermelon and melon extracts (tested at a concentration between 1 ng/ml and 10  $\mu$ g/ml) using CD63 as the most common degranulation marker. BAT was positive for all concentrations tested (Supplementary Figure S1).

Next, we performed a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 15% acrylamide, 2.6% acrylamide–bis-acrylamide cross-linking) and IgE immunoblotting assay with patient serum (1:10 dilution). Two bands were detected with IgE reactivity: a band at 37-50 kDa in the watermelon extract, and (other band in the melon extract lower 37 kDa (Figure 1B). This band could correspond to a previously described NH<sub>2</sub>-terminal cucumisin fragment in this fruit [4]. While the protein identified in watermelon did not correspond to any allergen previously described.

To identify these proteins, watermelon extracts were concentrated in 10 kDa spin filter devices and analyzed by two-dimensional (2-D) gel electrophoresis using a pH gradient of 4 to 7 in the first dimension (a first approach was performed with a pH gradient of 3-10 to rule out the presence of basic proteins.). After second dimension on SDS-PAGE (12% acrylamide, 2.6% acrylamide–bis-acrylamide cross-linking), one gel was transferred onto a nitrocellulose membrane and another was stained with Coomassie blue. The blotted membrane was incubated with watermelon sensitive patient serum

(1:10 dilution) and developed with anti-human IgE secondary antibody. Three proteins, with a molecular weight between 37 and 50 kDa and isoelectric point (pI) between 5.1 and 5.6, were recognized by serum IgE antibodies (Figure 1C).

To identify these IgE-reactive proteins, spots matched between the immunoblot (Figure 1C) and the Coomassie blue-stained 2-D gel (Figure 1D) were manually excised (gel pieces) from the stained gel and processed for identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS). Gel pieces were incubated for in-gel digestion performed as described previously with minor modifications [5]. Finally, tryptic peptides were collected for peptide mass fingerprinting (PMF) analysis by MALDI-TOF MS [8]. MS data from PMFs spectra were searched in the NCBI database using the Mascot database search algorithm for protein identification. Two different protein were identified: (i) alpha-galactosidase of *Cucumis sativus* (spots 1 and 3 which correspond to the same protein), and (ii) luminal-binding protein, also named as 78 kDa glucose-regulated protein, GRP-78, of *Cucumis melo* (spot 2). These proteins are not present in *Citrullus lanatus* in the NCBI database and there are no reports of allergic reactions. The table of the identifications are shown in the Supplementary Material (Table S1).

The allergenic implication of GRP-78 has been reported, identifying GRP-78 in several plants as potential responsible of part of the cross-reactivity between proteins from different pollen and plant foods, like Anacardiaceae species, with 88-92% sequence identity in different vegetables but none in Cucurbitaceae family [6]. In 2013 Nayak et al. identified and characterized allergens from *Cannabis sativa* in patients sensitized to this plant, and observed that GRP-78 was implicated in the allergic response [7].

Alpha-galactosidases have been described in *Cucumis sativus* and *Cucumis melo*, and are widely present in other vegetal species, microorganisms and animals. They belong to a glycoside hydrolases family that can catalyze the release of  $\alpha$ -D-galactosyl substituents from sugars such as galacto-oligosaccharides, galactomannans and galactolipids [8]. In the pharmacological field, it has been reported cases of anaphylaxis-IgE-related in patients with Fabry's disease treated with recombinant alpha-galactosidase (agalactidase beta) [9]. Other hydrolases like beta-glicosidases have been recently proposed as major native allergen between Mediterranean pollen trees *Cupressus arizonica* and *Olea europea* [10].

To our knowledge, we report a systemic IgE-mediated reaction due to watermelon with alpha-galactosidase and GRP-78 as novel implicated allergens in this fruit. GRP-78 has been reported in allergy to Anacardiaceae and *Cannabis sativa* [6,7], however it is the first report of its implication in fruit allergy such as watermelon. This case illustrates the heterogeneity of food allergy and how in-depth study of food allergy reactions can lead to new diagnoses.

### **Conflicts of interest statement**

Carpio-Escalona LV, Fernández-Lozano C, Peracho L, Martínez-Alonso E, Rita CG, Martínez-Botas J, Alcázar A, and de la Hoz Caballer B have no conflict of interest to declare.

### **Funding sources**

Emma Martínez-Alonso thanks the MICINN for the contract PTA2017-14093-I.

## References

1. Rodríguez Del Río P, Díaz-Perales A, Sánchez-García S, Escudero C, Ibáñez MD et al. Profilin, a Change in the Paradigm. *J Investig Allergol Clin Immunol*. 2018;28(1):1-12.
2. Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M et al. Identification of major allergens in watermelon. *Int Arch Allergy Immunol*. 2009;149(4):291-8.
3. González-Mancebo E, López-Torrejón G, González de Olano D, Santos S, Gandolfo-Cano M, Meléndez A, et al. Identification of potential allergens involved in systemic reactions to melon and watermelon. *Ann Allergy Asthma Immunol*. 2010;104(3):271-2.
4. Cuesta-Herranz J, Pastor C, Figueredo E, Vidarte L, De las Heras M, Durán C, et al. Identification of Cucumisin (Cuc m 1), a subtilisin-like endopeptidase, as the major allergen of melon fruit. *Clin Exp Allergy*. 2003;33(6):827-33.
5. Martínez-Alonso E, Alcázar P, Camafeita E, Fernández-Lucas M, Ruíz-Roso G, Alcázar A. Proteomic analysis of plasma proteins of high-flux haemodialysis and on-line haemodiafiltration patients reveals differences in transthyretin levels related with anaemia. *Sci Rep*. 2020;10(1):16029.
6. Bastiaan-Net S, Reitsma M, Cordewener JHG, van der Valk JPM, America TAHP, Dubois AEJ, et al. IgE Cross-Reactivity of Cashew Nut Allergens. *Int Arch Allergy Immunol*. 2019;178(1):19-32.
7. Nayak AP, Green BJ, Sussman G, Berlin N, Lata H, Chandra S, et al. Characterization of Cannabis sativa allergens. *Ann Allergy Asthma Immunol*. 2013;111(1):32-7.

8. Zhang ZP, Liu YC, Dai HB, Miao MM. Characteristics and expression patterns of six  $\alpha$ -galactosidases in cucumber (*Cucumis sativus* L.). *PLoS One*. 2021; 16(1):e0244714.
9. Bodensteiner D, Scott CR, Sims KB, Shepherd GM, Cintron RD, Germain DP. Successful reinstatement of agalsidase beta therapy in Fabry disease patients with previous IgE-antibody or skin-test reactivity to the recombinant enzyme. *Genet Med*. 2008 May;10(5):353-8.
10. Bistoni O, Emiliani C, Agea E, Russano AM, Mencarelli S, Orlacchio A, et al. Biochemical and immunological characterization of pollen-derived beta-galactosidase reveals a new cross-reactive class of allergens among Mediterranean trees. *Int Arch Allergy Immunol*. 2005;136(2):123-33.

**Figure 1.** Detection of IgE-reactive proteins from serum of watermelon-sensitive patient. A, Slot Blot. IgE binding to watermelon, melon, *Phleum pratense* and *Platanus acerifolia* extracts and PBS as negative control. B, western blot of watermelon and melon extracts (~5 µg). C, western blot performed after two-dimensional (2-D) gel electrophoresis analysis of watermelon extract (~100 µg). D, watermelon extract (~100 µg) analyzed by 2-D gel electrophoresis and stained with Coomassie blue. Protein spots marked 1 to 3 were matched between the western blot membrane (C) and the stained 2-D gel (D), and were then excised and processed for MALDI-TOF MS identification. MW, molecular weight of protein markers in kDa.

