Two New Allergens in Watermelon (*Citrullus lanatus*) Allergy

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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 between these fruits is widely known. Few cases of allergic reactions to watermelon have been reported. Oral allergy syndrome is the main clinical manifestation, with systemic reactions being less frequent [1]. In 2009, Pastor et al [2] reported the 3 major allergens in watermelon to be malate dehydrogenase (36 kDa), triosephosphate isomerase (28 kDa), and profilin (13 kDa), with frequencies of sensitization of 96%, 78%, and 56%, respectively. The most recent case involved cucumisin (55 kDa) and phloem lectin Lec 17-1 (18 kDa) and cross-reactivity with melon [3].

We report the case of a 54-year-old man with grass pollen–induced allergic rhinoconjunctivitis who developed dysphonia, oropharyngeal and palmoplantar pruritus, and genital swelling with no other symptoms about 3 hours after the ingestion of a piece of watermelon and a few minutes after the ingestion of dates (*Phoenix dactylifera*). The patient recovered with dexchlorpheniramine and methylprednisolone. In the following 2 weeks, he developed the same symptoms in 2 mild episodes 15 minutes after repeated watermelon consumption. He subsequently tolerated tree nuts, other fruits including melon, and cucurbits.

Skin prick tests (SPTs) were performed with commercial extracts of watermelon, melon, *Phleum pratense*, *Platanus acerifolia*, palm pollen profilin (Pho d 2), peach lipid transfer protein (LTP), dates, and tree nuts including cashew. The results were positive for *P pratense*, *P acerifolia*, Pho d 2, watermelon, and melon. Prick-by-prick testing with watermelon and melon pulp yielded positive results, as did SPT with protein extract from pulp and the inner part of the rind of both fruits.

Total serum IgE was 29 kU/L, with negative results for watermelon and melon-specific IgE (0.04 kU/L and 0.06 kU/L, respectively) and positive results for Phl p 1 + Phl p 5b (3.98 kU/L) and Phl p 12–specific IgE (0.44 kU/L). Testing was performed using the UniCap System (Phadia), although the results were not available for Pho d 2 in the case we report. A controlled oral challenge test with dates yielded negative results.

Protein extracts from watermelon and melon were prepared by homogenization in 20% wt/vol phosphate-buffered saline. 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-µm filter pore diameter). The allergenic potential of watermelon and melon extracts was confirmed by slot blot to preserve allergen integrity (Figure, A).

A basophil activation test (BAT) was performed with watermelon and melon extracts (tested at between 1 ng/mL and 10 µg/mL) using CD63 as the most common degranulation marker. The BAT result was positive for all concentrations tested (Supplementary Figure S1).

Next, we performed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE]) (15% acrylamide, 2.6% acrylamide–bis-acrylamide cross-linking) and IgE immunoblotting assay with the patient's serum (1:10 dilution). IgE reactivity was detected for 2 bands: a 37- to 50-kDa band in the watermelon extract and a <37-kDa band in the melon extract (Figure, B). This band could correspond to a previously described NH2-terminal cucumisin fragment in watermelon [4]. The protein identified in watermelon did not correspond to any previously described allergen.

To identify these proteins, watermelon extracts were concentrated in 10-kDa spin filter devices and analyzed using 2-dimensional gel electrophoresis with 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 the 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 antihuman IgE secondary antibody. Three proteins with a molecular weight between 37 and 50 kDa and isoelectric point between 5.1 and 5.6 were recognized by serum IgE antibodies (Figure, C).

To identify these IgE-reactive proteins, spots matched between the immunoblot (Figure, C) and the Coomassie blue–stained 2-dimensional gel (Figure, D) 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, which was performed as described previously with minor modifications [5]. Finally, tryptic peptides were collected for peptide mass fingerprinting analysis by MALDI-TOF MS [8]. MS data from the peptide mass fingerprinting spectra were searched for in the NCBI database using the Mascot database search algorithm for protein identification. Two different proteins were identified, as follows: (*i*) α-galactosidase of *Cucumis sativus* (spots 1 and 3, which corresponded to the same protein); and (*ii*) luminal binding protein, also known as 78-kDa glucose-regulated protein, GRP-78, of *C* (spot 2). These proteins are not present in *C lanatus* in the NCBI database, and there are no reports of allergic reactions. The identifications are shown in the Supplementary Material (Table S1).

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

α-Galactosidases have been described in *C sativus* and *C melo* and are widely present in other vegetable species, microorganisms, and animals. They belong to a glycoside hydrolase family that can catalyze the release of α-D-galactosyl substituents from sugars such as galacto-oligosaccharides, galactomannans, and galactolipids [8]. Cases of IgE-related anaphylaxis have been reported in patients with Fabry disease treated with recombinant α-galactosidase (agalsidase ß) [9]. Other hydrolases, such as ß-glycosidases, have recently been proposed as major native allergens in pollen from the Mediterranean trees *Cupressus arizonica* and *Olea europaea* [10].

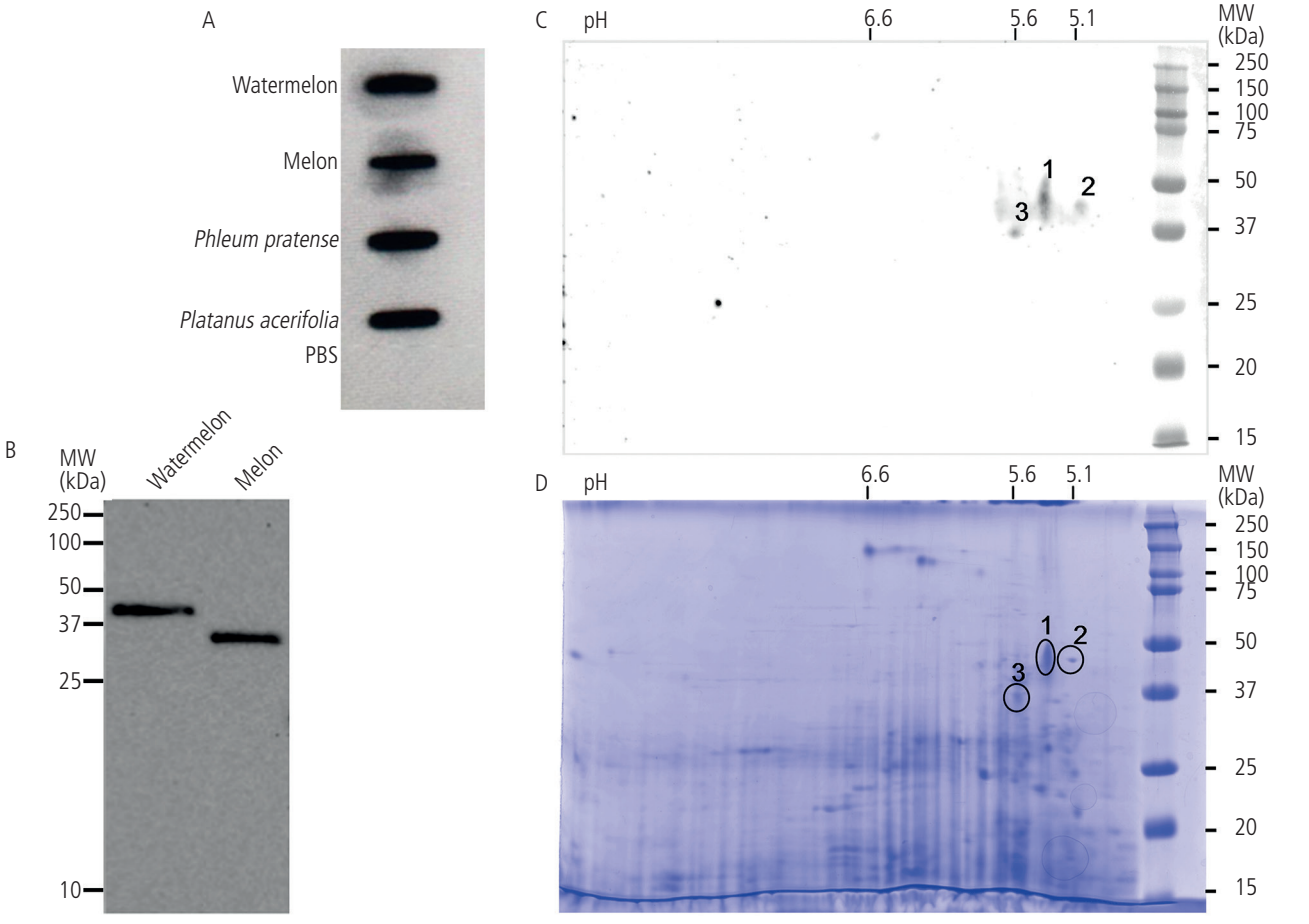

Figure. Detection of IgE-reactive proteins from the serum of a watermelon-sensitive patient. A, Slot blot. IgE binding to watermelon, melon, *Phleum pratense*, and *Platanus acerifolia* extracts and phosphate-buffered saline as a negative control. B, Western blot of watermelon and melon extracts (~5 μg). C, Western blot performed after 2-dimensional gel electrophoresis analysis of watermelon extract (~100 μg). D, Watermelon extract (~100 μg) analyzed using 2-dimensional 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-dimensional gel (D) and were then excised and processed for identification using MALDI-TOF MS. MW indicates molecular weight of protein markers in kDa; PBS, phosphate-buffered saline.

We report a systemic IgE-mediated reaction to watermelon, with α-galactosidase and GRP-78 as novel implicated allergens in this fruit. GRP-78 has been reported in allergy to Anacardiaceae and *C sativa* [6,7]. The case we report is the first in which it was involved in allergy to fruit, such as watermelon. This case illustrates the heterogeneity of food allergy and how in-depth study of allergic reactions to food can lead to new diagnoses.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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