Lipid transfer proteins and thaumatins as relevant allergens in melon peel allergy

Melon (*Cucumis melo*) belongs to the Cucurbitaceae family and is one of the most frequently involved fruits in allergic reactions. Oral allergy syndrome after ingestion of pulp is the main manifestation; systemic reactions are rare.[1] Several allergens have been identified in pulp, as follows: cucumisin (Cuc m 1),[2] profilin (Cuc m 2),[3] pathogenesis-related protein (PR) of the PR-1 family (Cuc m 3),[4] malate dehydrogenase,[5] and phloem lectin (Lec 17-1). We report a patient with allergic symptoms after ingestion of melon pulp and after contact with melon peel.

A 15-year-old girl experienced oral pruritus and labial angioedema while eating melon. She also presented palpebral angioedema and conjunctivitis in the eye she touched after physical contact with melon peel. She had presented labial angioedema immediately after eating an omelet made of peeled zucchini, although she subsequently tolerated eggs and other Cucurbitaceae (peel and pulp of water melon, cucumber, and pumpkin). Parental consent was provided for an allergy workup.

The results of skin prick tests with commercial extracts of melon, palm profilin, and lipid transfer protein (LTP) of peach (Pru p 3) were negative. Skin prick tests with standardized extracts of common aeroallergens (ALK-Abelló S.A., Madrid, Spain) were positive (wheal, >3 mm) for pollens (grass and *Olea europaea*). Prick-by-prick testing with melon was positive for pulp (5 mm) and very positive for peel (30 mm). The results of prick-by-prick testing to other Cucurbitaceae peels were slightly positive (watermelon, cucumber, and pumpkin), even though the patient had always tolerated them. Of the pulps tested, only that of zucchini gave a positive result. Serum specific immunoglobulin E (IgE) was positive to cucumber (1.22 kU_A/L) but not to melon. Total IgE was 62.8 IU/mL. The melon peel extract was manufactured by homogenization in phosphate-buffered saline, dialyzation, and lyophilization. Sodium dodecyl sulfate polyacrylamide gel electrophoresis IgE-immunoblotting assay with this extract revealed IgE reactivity bands of 7 to 8 kDa and 28 to 29 kDa (Fig 1). To identify these IgE-binding proteins, both bands from the melon peel extract were manually excised from the gel, digested with trypsin, and analyzed using matrix-assisted laser desorption/ionization-time of flight and liquid chromatography electrospray ionization tandem mass spectrometry following the methodology of Pastor et al.[6] Proteins were identified by searching a nonredundant protein sequence database (NCBI) using the Mascot program (http://www.matrixscience.com). We performed MS/MS and obtained 2 sequences of internal peptides: SLNQAATTADRR for the 7- to 8-kDa protein (M2) and SGPGQPQLSTTGFELAR-SFTVPAPWTGR for the 28- to 29-kDa protein (M1). Research conducted with protein databases identified the first sequence as an LTP and the second as the thaumatin-like protein from *Cucumis melo* (Gi: 38606865). To confirm the absence of cross-reactivity between LTP from melon and peach, the patient's serum was pre-incubated with peach extract. Immunoblotting inhibition assays were performed after incubation of 1 mL of serum with 100 μg of melon peel or peach peel extract at room temperature for 4 hours with constant stirring. IgE binding was not inhibited in the serum with the peach peel extract, although it was totally inhibited in the serum with the melon peel extract. In addition, immunoblotting assay using anti–Pru p 3 in melon peel extract did not recognize any bands. Immunoblotting with melon pulp to determine whether these new LTPs and thaumatin were also present did not reveal IgE-binding bands (polyclonal anti–Pru p 3 was donated by Dr. Díaz-Perales; UPM-INIA, Madrid, Spain). Previous tests with polyclonal antibodies showed extremely small amounts of protein (nanograms) in immunoblotting assays. Therefore, the lack of IgE binding was probably attributable to the absence of these proteins in the pulp extract.

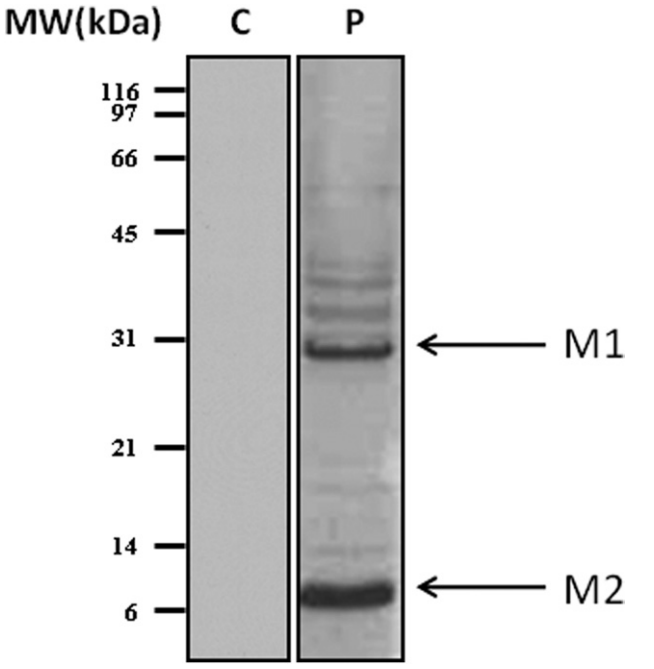

Fig. 1. Identification of allergens in melon peel extract. Sodium dodecyl sulfate polyacrylamide gel electrophoresis immunoblotting with melon peel extract in solid phase. *Lane P:* Incubated with patient serum; *Lane C:* Incubated with control serum (pool of sera from nonatopic subjects). Abbreviation: MW, molecular weight marker in kDa.

The LTPs, which belong to the PR-14 family, are very highly conserved and have a molecular weight of approximately 9 kDa. They are usually involved in systemic allergic reactions caused by vegetables, fruits (eg, Rosaceae), nuts, cereals, latex, and pollens. Although cross-reactivity has been described within the LTP family, homology between LTP sequences from botanically unrelated vegetable foods varies from 35% to 95%[7]. Consistent with our findings, some nonspecific LTPs do not cross-react with Pru p 3.[8] Thaumatins are very stable proteins belonging to the PR-5 family. They have a molecular weight of approximately 23 kDa and are involved in systemic allergic reactions to fruits, vegetables, and pollens.

Allergy to melon pulp has been reported in patients with pollinosis who are sensitized to profilin,[1] although no data exist for allergy to melon peel. We present a case of IgE-mediated contact angioedema to melon peel involving an LTP and a thaumatin-like protein precursor (*Cucumis melo*) as relevant allergens. The absence or scarce presence of these proteins in pulp could explain why we were unable to find them. Although allergic reactions to melon are usually attributed to pulp allergens, we should also take into account the allergens present in peel.

M. Gandolfo-Cano, MD*
E. González-Mancebo, MD, PhD*
D. González-de-Olano, MD*
E. Mohedano-Vicente, MD*
E. Muñoz-Garcia, MD†
B. Bartolomé, PhD‡
C. Pastor-Vargas, PhD†

Disclosures: Authors have nothing to disclose.

*Allergy Unit
Hospital Universitario de Fuenlabrada
Madrid, Spain
†Instituto de Investigaciones Sanitarias
Fundación Jiménez Díaz
Madrid, Spain
‡Reserch & Development Department
Bial-Arístegui
Bilbao, Spain
mgandolfo.hflr@salud.madrid.org

References

[1] Figueredo E, Cuesta-Herranz J, De-Miguel J, et al. Clinical characteristics of melon (Cucumis melo) allergy. *Ann Allergy Asthma Immunol*. 2003;91:303–308.
[2] Cuesta-Herranz J, Pastor C, Figueredo E, et al. Identification of Cucumisin (Cuc m 1), a subtilisin-like endopeptidase, as the major allergen of melon fruit. *Clin Exp Allergy*. 2003;33:827–833.
[3] López Torrejón G, Crespo JF, Sánchez-Monge R, et al. Allergenic reactivity of the melon profilin Cuc m 2 and its identification as major allergen. *Clin Exp Allergy*. 2005;35:1065–1072.
[4] Asensio T, Crespo JF, Sánchez-Monge R, et al. Novel plant pathogenesis-related protein family involve in fruit allergy. *J Allergy Clin Immunol*. 2004;114:896–899.
[5] González-Mancebo E, López-Torrejón G, González-de-Olano D, et al. Identification of potential allergens involved in systemic reactions to melon and watermelon. *Ann Allergy Asthma Immunol*. 2010;104:271–272.
[6] Pastor C, Cuesta-Herranz J, Cases B, et al. Identification of major allergenes in watermelon. *Int Arch Allergy Immunol*. 2009;149:289–290.
[7] Asero R, Mistrello G, Roncarolo D, et al. Immunological cross-reactivity between lípid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy*. 2002;57:900–906.
[8] Tordesillas L, Sirvent S, Díaz-Perales A, et al. Plant lipid transfer protein allergens: no cross-reactivity between those from foods and olive and Parietaria pollen. *Int Arch Allergy Immunol*. 2011;156:291–296.

Hospital admissions for allergy and eczema varied across regions in England, 2008–2011

Allergic disorders are common throughout the United Kingdom, affecting males and females of all ages and peoples from all social classes and ethnic groups. In the 1990s, the largest increases in rates have been for anaphylaxis and food allergy. Anaphylaxis rates rose from 6 to 41 per million between 1990–1991 and 2000–2001, and food allergy rates rose from 5 to 28 per million over this period.[1] Moreover, direct National Health Service costs for managing allergic problems were estimated at over 1 billion UK pounds per annum, and treatments for asthma and other allergic disorders have accounted for 10% of primary care prescribing costs.[1] In terms of population prevention, avoiding preventable hospital admissions would be an effective strategy. Therefore, I investigated recent hospital admissions for all allergies in England.

Hospital Episode Statistics (HES) between July 1, 2008 and June 30, 2011 were obtained from Lightfoot Solutions, using the sfn system (http://www.lightfootsolutions.com), which enables access to real-time HES data.[2] The HES database contains information about all patients admitted to National Health Service hospitals in England.[3] It was first established in 1989 and currently collects data on more than 12 million hospital admissions each year. No apparent barriers to access exist because all UK residents are entitled to primary care consultations, which are free at the point of use, and most residents are registered with a practice. The HES online system with complete information is available from 1997/1998, and the dataset contains many variables, including age, sex, primary and secondary diagnoses, and in-hospital death.[3] The basic unit of the dataset is the consultant episode, covering the continuous period during which a patient was under the care of 1 consultant. Episodes of care were linked into admissions, and those ending in transfer to another hospital were linked together to avoid multiple counting.[4] The main reason for admission ("primary diagnosis") is coded using the *International Classification of Diseases,* version 10 (ICD-10) codes.[5] In the current study, J30.1, J30.2, J30.3, J30.4, J45.0, K52.2, L50.0, L56.1, T78.0, T78.1, T78.2, T78.4, T80.5, and T88.6 were used for accounting allergies admissions.[3] In addition, 2010 mid-year population estimates for year and age were obtained from the UK National Statistics (http://www.statistics.gov.uk).[4] Moreover, because deprivation in particular has been shown to play a major role in risk of hospital admissions,[5] it[6] (including 7 domains such as income, employment, health and disability, education and skill, barriers to housing and services, crime, and living environment) was therefore included for comparisons. All graphs were produced using Excel (Microsoft Corp, Redmond, Washington).

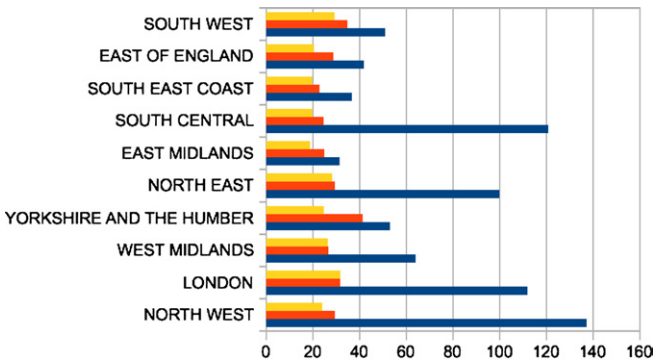

Fig. 1. Hospital admissions for all allergies by regions (top to down, showing regions with 20% most deprivation: the lowest to the highest).

In the northwest (137.20 per 100,000) and south central (120.73 per 100,000) regions (Fig 1), apparent peaks in allergy admissions were seen for those younger than 15 years, followed by London (111.85 per 100,000) and the northeast (99.81 per 100,000). Conversely, in London (31.67 per 100,000), the southwest (29.25 per 100,000) and northeast (28.17 per 100,000), higher allergies admissions were observed for those older than 76 years old. For adults aged between 16 and 75 years, generally similar allergies admissions patterns were found across regions, with a slightly higher rate in Yorkshire (41.27 per 100,000) and the southwest (34.72 per 100,000). Table 1 shows average annual hospital admissions rates for allergic diseases in the United Kingdom from July 1, 2008 to June 30, 2011 by age group and sex per 100,000 population (all admissions divided by 3). Interestingly, asthma admissions and other related admissions were all higher in males among those younger than age 15 but lower than female in those aged 16 and older.

Strengths of this study lie in its large nationally representative population-based sample and provision of recent clinical situations. Although the accuracy of HES depends on clinical diagnoses recorded in patients' medical records and may lead to misclassification bias, in recent years the quality of coding has improved, and HES data have been of good quality and used extensively for re-

Disclosures: Authors have nothing to disclose.
Funding Source: I.S. is supported by European Regional Development Fund and the European Social Fund. Convergence Programme for Cornwall and the Isles of Scilly.