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Pomegranate allergy and pathogenesis-related protein 4

Pomegranate is the fruit of *Punica granatum*, a tree native to the Himalayas in northern India and widely cultivated throughout the Mediterranean region.¹ However, there are not many published cases describing immediate hypersensitivity reactions after ingestion.^{1–3} Recently, pomegranate was shown to contain 2 protein allergens, of 29 kDa and 9 to 12 kDa, the latter of which was reported to be a lipid transfer protein (LTP).⁴ Herein, we report a patient with a severe systemic reaction after consumption of pomegranate.

A 5-year-old boy was admitted to the emergency room with urticaria, pruritus, wheezing, and shortness of breath (anaphylaxis) 10 minutes after ingestion of pomegranate. He was administered intramuscular adrenaline, oral antihistamine, methylprednisolone, and salbutamol via nebulizer, and his clinical symptoms improved within an hour. When he was 6 months old he had been diagnosed with milk and eggwhite allergies, and at 3, with hazelnut and pistachio allergies.

His family indicated that he had eaten pomegranate for the first time 5 months ago and symptoms of urticaria, pruritus, and dyspnea had developed. Skin prick test with fresh pomegranate using a prick-to-prick method was positive (wheal diameter 6 mm), and skin prick tests with commercial extracts to milk, egg, hazelnut, peanut, pistachio, and grass pollen were also positive. Serum specific immunoglobulin E (IgE) levels to extracts from hazelnut (89.9 kU/L), peanut (1.6 kU/L), pistachio (16.5 kU/L), pomegranate (0.6 kU/L), and purified peach LTP (Pru p 3) (2.7 kU/L) were detected by Enzym Allergo Sorbent Test technique.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting assay^{5,6} was performed under nonreducing electrophoretic conditions to study the possible implication of the pomegranate LTP in the allergy reaction. A sample of purified Pru p 3 was studied, and the apparent molecular mass of an LTP protein turned out to be 16 kDa. Two pomegranate IgE binding bands were found with the patient serum; their molecular masses were 16 kDa and 17 kDa, and an anti-Pru p 3

rabbit serum revealed 3 IgE binding bands of 14.5 kDa, 17.5 kDa, and 28 kDa (Fig 1).

Although nonreducing conditions may be an explanation for the apparent molecular mass detected for the tentative pomegranate LTP proteins (higher than that indicated by the LTP sequences),⁴ we further carried out a proteomic assay to identify the pomegranate IgE-binding bands. Two-dimensional gel electrophoresis and identification of proteins by tandem mass spectrometry was performed as described previously.⁷ Protein identification was performed by searching a nonredundant protein sequence database (National Center for Biotechnology Information) using the Mascot program (<http://www.matrixscience.com>).

The proteomic analysis indicated that the IgE-binding proteins from pomegranate were not LTP proteins; they were pathogenesis-related (PR) 4 proteins that belong to the Barwin family. We found 100% identity between a peptide from these pomegranate proteins and peptides from PR-4 proteins from the genera *Prunus*, *Malus*, and *Chimonanthus*. We also found 81% identity between another peptide from these proteins and a peptide from a chitin-binding protein from *Brassica rapa*, the allergen Bra r 2, and peptides from PR4 proteins from the genus *Vitis*.

The PR proteins have been defined as plant proteins that are induced on pathogen attack and stress. Of the 17 groups of PR proteins, the allergenic character of groups 2, 3, 4, 5, 8, 10, and 14 have been demonstrated. Their particular features, such as low molecular weight, stability at low pH, and resistance to proteolysis, enable them to induce immune responses.⁸ PR-4 type proteins are chitinases and share a common C-terminal Barwin domain. They were first described in potato win 1 and win 2 proteins, which have a high sequence similarity with prohevein, and later were isolated from many other plants (eg, tomato, tobacco, *Arabidopsis*, rubber tree plants [*Hevea brasiliensis*], wheat, apple [*Malus domestica*], barley, and maize). Prohevein and Bra r 2 (*Brassica rapa*) from the PR-4 family exhibit allergenic activity.⁹ Prohevein (Hev b 6.01) was identified as a major allergen from *Hevea brasiliensis* latex and has 2 domains, hevein (Hev b 6.02) and the C-terminal domain (Hev b 6.03). IgE binding to Hev b 6 is found in 75% to 83% of latex-allergic patients. Also, Hev b 6.03 is shown to have homology to PR-4

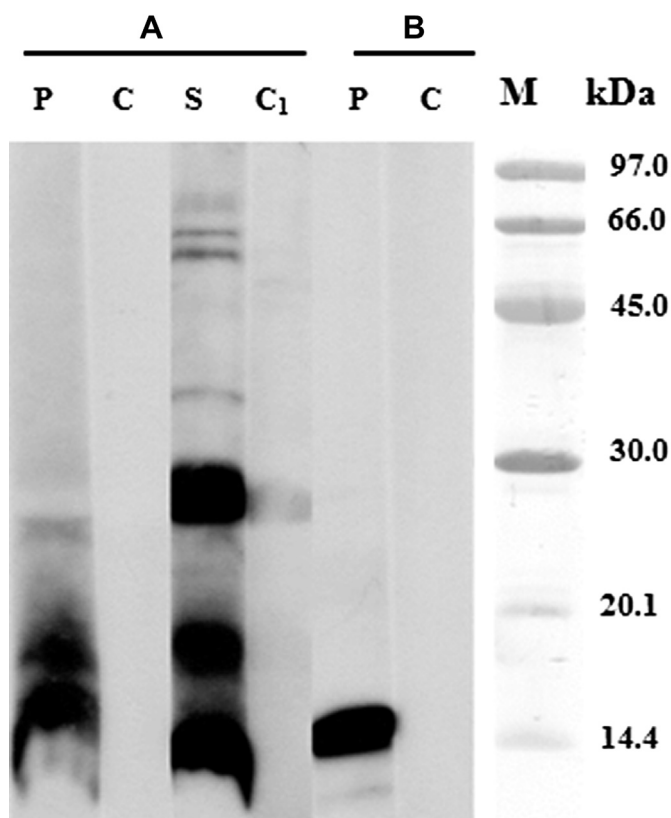


Figure 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting: A, Extract from pomegranate (pulp). B, Peach LTP (Pru p 3). Lane P: Patient serum; Lane C: Control serum (pool of sera from nonatopic subjects); Lane S: Anti-Pru p 3 rabbit serum; Lane C1: Nonimmunized rabbit serum.

proteins from tobacco, potato, and soybean. Turnip has been shown to express an 18.7 kDa allergen (Bra r 2) after wounding or chemical treatment, which turned out to be IgE-reactive to latex-allergic patient sera. This turnip allergen has 70% identity to Hev b 6.01, besides high sequence similarity to wound-induced proteins from tomato (74%) and potato (71%).¹⁰

This case has paramount importance because of 2 features: Most cases of pomegranate allergy have been reported from Spain and Italy, and this case is the first to be presented from Turkey. Although the relationship between pomegranate allergy and LTPs has been

demonstrated previously, this is the first time PR-4 proteins have been described as pomegranate allergens. We suggest that pomegranate should be considered as a potential allergen in Mediterranean countries, and PR-4 proteins should be taken into account as a relevant allergen along with the LTPs (PR-14) in pomegranate allergy.

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