

We focused this study on the 17-kDa protein because it was the ferret urine protein with the highest IgE-binding capacity. This band was visualized in 2-dimensional immunoblots to specify the proteins contained. The isoelectric point was 5, detected using the 2-dimensional immunoblotting technique. The 17-kDa band was analyzed by means of matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MS) and liquid chromatography electrospray ionization tandem MS following the method of Gallego-Delgado et al.² The analysis of the resulting peptides using MS or MS/MS did not correspond to any previously reported allergens compared with the databases (Fig 1B). To purify the 17-kDa protein, male ferret urine was dialyzed against 20-mmol/L Tris-hydrochloride, pH 7.4, loaded on a Mono Q (GE Healthcare). The allergen was eluted with a 1M sodium chloride lineal gradient. The SPT was performed with this purified allergen with positive results. Immunoblotting with the patient's serum was performed with this purified protein (Fig 1A). Basophil activation assays using the 17-kDa purified protein were performed with the patient's heparinized whole blood (BASOTEST; ORPEGEN Pharma, Heidelberg, Germany), with concentrations ranging from 0.001 to 50 µg/mL, obtaining positive results according to the manufacturer.

Pets are usually a source of potent allergens and are permanent inhabitants of houses. Ferrets are related to minks, skunks, weasels, otters, and badgers, but they are the only domestic species in the Mustelidae family. Few cases of allergy to ferret have been reported. The capacity of these animals as a relevant source of allergens has not been extensively studied; Codina et al³ demonstrated the presence of 4 allergens in male ferret urine (103, 81, 28.8, and 14.8 kDa) and 1 in ferret pelt (81 kDa). IgE-binding bands with similar molecular weight have been described from other mammal sources.^{3,4} Nugent et al⁵ described a 66-kDa protein with in vitro IgE-binding capacity from ferret hair and urine, suggesting that albumin is the relevant allergen.

In this study, clinical history and detection of specific IgE antibodies against FEs suggested an IgE-mediated allergic reaction. Immunoblotting revealed IgE reactivity with 59-, 34-, 25-, and 17-kDa proteins. Inhibition assays showed cross-reactivity between extracts from male ferret urine and dog dander and cat epithelium. The MS assays performed with the 17-kDa purified protein did not match with previously reported sequences. The positive results obtained with purified 17-kDa ferret protein, SPT, and basophil activation assay demonstrated the allergenic nature of this protein.

We presented a case of allergy to ferret involving a 17-kDa allergenic protein that has not been previously reported. Because ferret is becoming a common pet and its allergenic nature has been proved, this report should be taken into account in future diagnostic and therapeutic research in ferret allergy.

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POMEGRANATE (*PUNICA GRANATUM*) ALLERGY: CLINICAL AND IMMUNOLOGICAL FINDINGS

Pomegranate is the fruit of the pomegranate tree (*Punica granatum*), a dicotyledon angiosperm plant of the Lythraceae family. The tree flowers in May and June, and its fruits mature in September and October. The pulp of the fruit consists of a cluster of fleshy, reddish violet seeds. Pomegranate fruits are commonly consumed in the Mediterranean areas, both raw and in processed forms, such as juice and flavorings. They have been infrequently reported to cause immediate hypersensitivity after ingestion.^{1,2} Pomegranate has already been reported to contain a 29-kDa protein and a 9- to 12-kDa protein, and characterization of the latter has confirmed that it is a lipid transfer protein (LTP).^{3,4} LTPs have been identified as major allergens in fruits belonging to the Rosaceae family. The wide distribution and the highly conserved structure of these proteins in various plant species confer these polypeptides a role as plant panallergens. In turn, LTPs are recognized as being responsible for immunological cross-reactivity among fruits, nuts, and/or pollens.⁵

To our knowledge, we present the first case series of patients with allergic symptoms that developed after eating fresh pomegranate fruit. The patients included the following: (1) a 19-year-old man who complained of 2 episodes of angioedema of the upper lips and tongue, which appeared a few minutes after ingestion of the fresh fruit; (2) a 13-year-old boy who had an episode of widespread urticaria and angioedema of the lips after ingestion of the fresh fruit; (3) a 31-year-old woman who complained of an episode of urticaria 20 minutes after ingestion of the fresh fruit; and (4) a 23-year-old woman who complained of an episode of angioedema of the face

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Table 1. Clinical Characteristics and Test Results of the Patients

Patient No.	Symptoms after pomegranate ingestion	Other symptoms	Skin prick tests								RAST, kU/L
			Common inhalant	Food commercial extracts	Pomegranate extracts						
					1:10,000	1:1,000	1:100	1:10	Undiluted		
1	Upper lip and tongue angioedema	Conjunctivitis, rhinitis, and asthma in spring and autumn; lip angioedema and oral itching after ingestion of walnut; eyelid edema and facial skin erythema after contact with a peach peel	Positive to ragweed pollen, olive tree, mimosa, cypress, <i>Alternaria</i> , and dog	Positive to soybean, hazelnut, peach, rice, pea, walnut, peanut, wheat, bean, almond, strawberry, and apple	Not tested	Neg	Pos (3 mm)	Pos (4 mm)	Pos (6 mm)	Peach, 0.37; walnut, 1.75; apple, 5.91; peanut, neg; and hazelnut, neg	
2	Lip angioedema and urticaria	Allergic oral syndrome after ingestion of walnut, celery, fennel, and peach	Positive to ragweed pollen, cypress, and grass pollen	Positive to peanut, orange, wheat, apple, hazelnut, walnut, rice, soybean, bean, peach, and pea	Pos (2 mm)	Pos (3 mm)	Pos (4 mm)	Pos (6 mm)	Not tested	Peach, 4.99; walnut, 0.47; apple, neg; peanut, neg; hazelnut, neg	
3	Urticaria	Spring rhinitis; and urticaria after taking ASA, almond, walnut, and peach	Positive to dust mite and olive pollen	Positive to peanut, orange, almond, apple, hazelnut, walnut, bean, and peach	Not tested	Pos (3 mm)	Pos (5 mm)	Pos (6 mm)	Not tested	Peach, 6.97; walnut, 1.4; and apple, 4.14	
4	Lip and face angioedema; generalized itching, including throat itch; and abdominal pain	Itching after ingestion of walnut, tuna, salmon, cherry, and lupine; ADR (ASA and antibiotics)	Negative	Positive to peanut, hazelnut, walnut, peach, soybean, rice, apple, and bean	Not tested	Neg	Neg	Pos (3 mm)	Pos (5 mm)	Peach, 6.95; walnut, 2.05; apple, 4.15; peanut, neg; and hazelnut, neg	

Abbreviations: ADR, adverse drug reaction; ASA, acetylsalicylic acid; neg, negative; pos, positive (diameter of the wheal); RAST, radioallergosorbent test; kU/L, kilounits/liter.

and lips, itching of the throat, abdominal pain, and widespread urticaria, which developed approximately an hour after ingestion of pomegranate fruit.

Table 1 gives the clinical characteristics of the patients and the tests used. To study these phenomena, pomegranate fruit proteins were extracted from pulp by stirring for 2 hours at 4°C in Björkstén buffer. Particles were removed by centrifugation at 15,000g for 30 minutes at 4°C, and the supernatant was dialyzed overnight against phosphate-buffered saline. The protein concentration of the extract (5.4 mg/mL) was determined using Bradford analysis, with bovine serum albumin as the standard.⁶ A sample of pomegranate extract was dissolved in sample buffer (Invitrogen, Milan, Italy) and β -mercaptoethanol at 100°C. Pomegranate proteins, 20 μ g, were separated by sodium dodecyl sulfate–polyacrylamide 12% separation gel electrophoresis (Nupage Bis-Tris, Invitrogen) at 200 V for 1 hour.⁷ A molecular weight markers kit was used as the protein standard. After separation, the gel was stained with 0.1% Coomassie brilliant blue. Separated protein bands were electrophoretically transferred to nitrocellulose membranes.⁸ The nitrocellulose membranes were saturated with 0.1-mol/L Tris-buffered saline and 0.5% fat-free milk powder and incubated overnight at 4°C with patients' serum samples. Bound specific IgE was detected by peroxidase-conjugated anti-human IgE antibodies from goat (1:10,000 in saturation buffer) (Sigma-Aldrich, Milan), using the chemiluminescence method.

All patients underwent skin prick tests with common inhalants and foods, including both the foods known to provoke symptoms and a standard panel, to elicit any other sensitizations and/or relate our data to those in the literature. Skin prick tests with pomegranate extracts were performed at increasing concentrations (from 1:10,000 to undiluted) until a wheal measuring 6 mm in diameter, the end point of our study protocol, was obtained. The starting dilution depended on the severity of the clinical manifestations.

As controls, 20 participants (10 atopic and 10 nonatopic) underwent prick testing with undiluted pomegranate extract, and all showed negative results.

Table 1 reports the results of the tests in the 4 allergic patients.

The gel profile of pomegranate extracts after staining with 0.1% Coomassie blue showed similar bands at 29 and 12 kDa, and immunoblotting analysis showed IgE reactivity of patients' serum to a single band at 29 kDa (Fig 1).

In this case series, the clinical data demonstrate that angioedema of the lips was present in 3 of the 4 patients (patients 1, 2, and 4), and 3 of the 4 patients (patients 2, 3, and 4) also developed urticaria. Two patients (patients 1 and 3) had a history of seasonal respiratory symptoms, and 3 (patients 1, 2, and 3) showed specific IgE to pollens (Table 1).

The symptoms and results after eating peaches and walnuts (Table 1) allowed us to classify these reactions as IgE mediated. In addition, the finding of specific IgE for apple in 3 of the 4 patients (patients 1, 3, and 4) with positive prick test results to the commercial extract of apple was interpreted as a purely in vitro phenomenon (all the patients were in the habit of eating apples with or without the peel without ill effects).

In this study, tests performed with diluted pomegranate extract and the results of immunoblotting led us to conclude that the manifestations were indeed IgE mediated and caused by the presence of specific IgE for the 29-kDa protein in all the patients' serum samples (Fig 1). Our case series warrants further in-depth study because the data emerging from the clinical histories and the tests

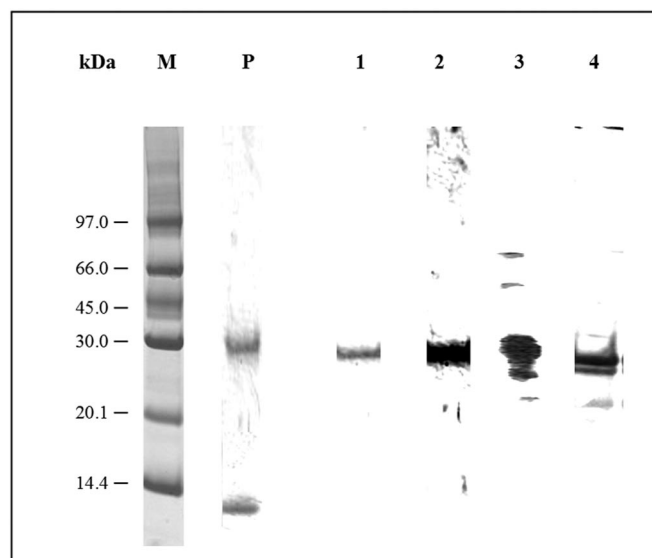


Figure 1. *Punica granatum* extract electrophoresis and immunoblotting results. Lane M indicates molecular mass markers; lane P, pomegranate extract gel electrophoresis results; and lanes 1, 2, 3, and 4, immunoblotting with serum samples from patients with a reaction after ingestion of *P granatum*.

performed also raise the suspicion of an oral allergic syndrome in patients allergic to pollens or of a possible association between hypersensitivity reactions and plant foods.

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