Letters

HLA CLASS II ALLELE AND IgG SENSITIZATION TO METHYLENE DIISOCYANATE IN EXPOSED WORKERS

Occupational asthma (OA) is characterized by airflow limitation and airway hyperresponsiveness caused by the occupational environment.[1] Isocyanates have been identified as the most common cause of OA.[2] In toluene diisocyanate–induced OA (TDI-OA), the HLA gene may play a role in susceptibility and protection against the disease.[3] There have been several reports of genetic relationships with HLA alleles in Italian[4,5] and Korean[6] populations; however, there are no published data on HLA allelic associations in methylene diisocyanate–induced OA (MDI-OA). We investigated associations among HLA alleles, specific antibody production, and MDI-OA phenotypes. The protocols were approved by the Ajou University institutional review board; informed consent was obtained from each participant.

Fifty-eight employees of a single car upholstery factory who were exposed to MDI participated in this study. Five workers had MDI-OA. The other 53 workers, who were exposed to MDI but did not have airway hypersensitivity, were enrolled as asymptomatic exposed controls (AECs). Serum specific IgE and IgG antibodies to MDI–human serum albumin (HSA) were detected by means of enzyme-linked immunosorbent assay. Lung function tests and inhalation challenge tests with methacholine and MDI were performed in individuals with respiratory symptoms to confirm the MDI-OA diagnosis.

Genomic DNA was extracted from each study sample, and allele-level genotypes of HLA class I (A, B, and Cw) and II (DRB1, DQB1, and DPB1) genes were obtained. Data analysis was performed using Sequence Navigator and MatchTool software (PE–Applied Biosystems Inc, Carlsbad, California), and statistical analyses were performed using SAS/Genetics software (SAS Institute Inc, Cary, North Carolina). Haplotype reconstruction was completed using PHASE. Hardy-Weinberg equilibrium for all loci and linkage disequilibrium for all pairs of loci were tested using Arlequin (v 2.0; Genetics and Biometry Laboratory, Geneva, Switzerland).

Participants with MDI-OA had significantly lower methacholine provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% values vs the AEC group (P = .007). There were no other significantly different clinical variables between groups (sex, age, atopy, exposure duration, smoking, total IgE concentration, lung functions, and specific antibodies to MDI-HSA). In the HLA analysis, the frequencies of A*2601 and Cw*0803 were significantly higher in individuals with MDI-OA compared with those in the AEC group (P = .031 and .019, respectively). In class II alleles, DRB1*0901 and DQB1*0303 were significantly prevalent in patients with MDI-OA than in AECs (P = .024 and .048, respectively). However, these statistically significant associations with HLA allele frequencies disappeared after performing Bonferroni multiple comparisons. In the haplotype frequency analyses, only B*4006-DRB1*0901, a 2-locus haplotype, was significantly prevalent in patients with MDI-OA vs AECs (P = .019). Compared with the single allele frequencies of the B*4006-DRB1*0901 haplotype, the frequency of B*4006 was not significant (P = .058) and of DRB1*0901 was significant (P = .024). When combined into a haplotype, B*4006-DRB1*0901, its frequency was significantly increased in patients with MDI-OA vs AECs (P = .019; odds ratio, 26.25), whereas no significant associations were found with other HLA class I or II alleles or other haplotypes. Table 1 summarizes the associations between HLA allele/haplotype frequencies and the presence of serum specific IgG antibodies to MDI-HSA conjugate. No single allele associations were observed on the presence of specific IgG antibodies; however, the 3-locus haplotype, DRB1*0901-DQB1*0303-DPB1*0501, was significantly associated with the presence of serum specific IgG antibodies (P = .035). No associations were observed regarding serum specific IgE antibody.

Genetic variants that determine susceptibility to specific environmental exposures may contribute greatly to the development of occupational diseases.[3] For TDI-OA, the pathogenic mechanism is unknown, and there have been several reports on the genetic susceptibility of Italian[4,5,7] and Korean[6] populations.

Although MDI is also a type of isocyanate, the pathogenesis of MDI-OA is not identical to that of TDI-OA. The results of a previous study suggest that the presence of MDI specific IgG antibodies might be predictive of MDI-OA.[8] Specifically, we investigated the possible association between HLA alleles and MDI specific IgG or IgE antibody production. We found that single allelic frequencies of DRB1*0901, DQB1*0303, and DPB1*0501 did not demonstrate significant associations with the presence of MDI specific IgG or IgE antibodies; however, the haplotype frequency DRB1*0901-DQB1*0303-DPB1*0501 was significantly associated with the presence of serum specific IgG to MDI-HSA conjugate. Therefore, we hypothesize that individuals with the DRB1*0901-

Table 1. Associations Between HLA Allele/Haplotype Frequencies and the Prevalence of Serum Specific IgG Antibodies to MDI-HSA Conjugate[a]

	Specific IgG to MDI-HSA conjugate, No. (%)		*P* value (OR)
	Presence	Absence	
DRB1*0901	6 (27.3)	9 (11)	.082
DQB1*0303	6 (27.3)	12 (14.6)	.204
DPB1*0501	10 (45.5)	28 (34.1)	.332
DRB1-DQB1-DPB1 0901-0303-0501	4 (18.2)	3 (3.7)	.035 (5.85)

Abbreviations: MDI-HSA, methylene diisocyanate–human serum albumin; OR, odds ratio.

[a] No associations were observed with respect to serum specific IgE antibody.

Disclosures: Authors have nothing to disclose.

Funding Sources: This study was supported by a grant from the Korean Health 21 R&D Project of the Ministry of Health & Welfare, Republic of Korea (A030001).

DQB1*0303-DPB1*0501 haplotype who are exposed to MDI have a higher probability of developing serum specific IgG to MDI, which could lead to a greater risk of MDI-OA.

This study is the first, to our knowledge, to examine genetic susceptibility in MDI-OA using HLA analysis. We suggest that the HLA allele DRB1*0901-DQB1*0303-DPB1*0501 may increase specific IgG sensitization, which could contribute to the development of the MDI-OA phenotype in exposed workers.

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MAST CELL DISEASES AND THE SEVERITY AND COURSE OF INTRAOPERATIVE ANAPHYLAXIS

It is well known that certain drugs may induce severe and fatal intraoperative anaphylactic reactions in patients with mast cell diseases.[1–3] Identifying these disorders is crucial for intraoperative patient management. According to some studies, medication seems to be the second cause of biphasic reactions. Unlike other risk factors for biphasic reactions (that include more rapid onset of anaphylaxis delayed use of epinephrine, low doses of epinephrine, etc), mast cell diseases have never been studied as such.[4] We describe 2 patients with intraoperative anaphylaxis who were diagnosed postoperatively as having mastocytosis.

Disclosures: Authors have nothing to disclose.

A 70-year-old woman was seen for a preoperative evaluation for malignant colon cancer. The patient had a history of polypharmacologic intolerance with pruritus caused by cyclooxygenase-1 inhibitors, cutaneous rush from an unspecified local anesthetic, and an episode of severe intraoperative anaphylaxis in 1983. Because her medical records were unavailable, allergy testing was performed as per the guideline protocol[5,6] for latex, mepivacaine, marcaine, lidocaine, suxamethonium, atracurium, cisatracurium, pancuronium, vecuronium, rocuronium, thiopentale, propofol, atropine, morphine, fentanyl, remifentanyl, chlorhexidine, polyvynilpyrrolidone, polygeline, major and minor antigenic determinants of penicillin, ampicillin, and piperacillin. All the results were negative. Two basal serum tryptase concentration measurements yielded 275 and 306 μg/L, respectively (reference range, $\leq$11.4 μg/L). Objective examination of the patient showed a few skin lesions, which were fixed, red-brown, and hyperpigmented. Histopathologic examination of the skin biopsy sample confirmed the diagnosis of urticaria pigmentosa. The patient refused a bone marrow biopsy to evaluate the presence of indolent systemic mastocytosis. Although drug desensitization may occur across time or we may not have tested for the offending drug administered in 1983, the final diagnosis was "probable non–IgE-mediated intraoperative anaphylaxis in a patient with urticaria pigmentosa." Although it is well known that mastocytosis can lead to the development of non–IgE-mediated severe intraoperative reactions, without standard guidelines for the intraoperative management of these patients, premedication consisted of antihistamines, corticosteroids, and disodium cromoglycate by means of nebulization and the oral route; stress-reducing ansiolitics were administered, and safer anesthetic drugs were used.[7,8] No undesired intraoperative or postoperative reactions occurred.

A 57-year-old woman was seen for an allergologic evaluation after severe intraoperative biphasic anaphylaxis. The second reaction (urticaria and hypotension) was less severe than the first and occurred 14 hours after resolution of the first episode. The patient had no history of previous drug-related reactions or atopy and had never had general anesthesia. Her medical record stated that she was administered fentanyl, remifentanyl, propofol, rocuronium, ampicillin, and ketorolac. Allergy testing, following the guidelines,[5] was performed for latex, atracurium, cisatracurium, pancuronium, vecuronium, rocuronium, suxamethonium thiopentale, propofol, atropine, fentanyl, remifentanyl, chlorhexidine, polyvynilpyrrolidone, and polygeline. All results were negative. Furthermore, CAP system results vs latex, suxamethonium, ethylene oxide, and polygeline were negative but vs penicilloyl G (1,410 U/mL), penicilloyl V (1,680 U/mL), amoxicillin (1,200 U/mL), and ampicillin (1,280 U/mL) were positive. The hypothesis was "IgE-mediated intraoperative anaphylaxis due to ampicillin." However, basal serum tryptase concentrations in 2 determinations were 36.3 and 42.0 μg/L. Histopathologic examination of the skin biopsy sample was negative for urticaria pigmentosa, whereas a bone marrow biopsy confirmed the presence of indolent systemic mastocytosis. The final diagnosis was "IgE-mediated intraoperative biphasic anaphylaxis due to ampicillin in a patient with indolent systemic mastocytosis."

In patients with severe intraoperative reactions, irrespective of the allergy test results, it is advisable to measure serum basal tryptase levels. In the presence of increased basal serum tryptase levels, patients should be evaluated for mast cell diseases. Such cases suggest that mast cell diseases could affect not only the severity of drug-induced anaphylaxis but also its course. Patients with mastocytosis should be informed of the risk factors involved and provided with medical alert identification describing their condition. In these

patients, a pluridisciplinary approach to intraoperative care and management among anesthetists, surgeons, and allergists is vital. Multicenter studies should be organized to evaluate the frequency of mast cell diseases or elevated basal tryptase levels in patients with a history of severe drug-induced anaphylaxis, irrespective of the presence of specific IgE, and to confirm the hypothesis that these disorders or elevated basal serum tryptase concentrations may represent a new potential risk factor for the development of biphasic anaphylaxis.

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ACKNOWLEDGMENT

We thank Paul Bowerbank for his help in reviewing the manuscript.

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HYPEREOSINOPHILIA: A CASE WITH AN ELUSIVE ETIOLOGY

A 48-year-old man residing in Iowa visited the emergency department with severe abdominal cramping, diarrhea, and an eosinophil count of 14,000/mm^3. Results of extensive evaluation, including abdominal/pelvic computed tomography, a complete metabolic panel, urinalysis, human immunodeficiency virus testing, and hepatitis B/C screening, were negative. His total IgE level was normal at 94 IU/mL. A stool study for ova and parasites was negative. An echocardiogram did not reveal any abnormalities. A colonoscopy with biopsies revealed small scattered lymphoid aggregates with no significant eosinophilia or parasites. Esophageal biopsy samples showed evidence of esophagitis and scattered intraepithelial lymphocytes with rare eosinophils. Duodenal biopsy samples were without evidence of organisms. A bone marrow biopsy sample had no evidence of FIP1L1-PDGFRA or JAK2 mutations but revealed hypercellular bone marrow with 13% eosinophils. Chest computed tomography revealed mediastinal lymphadenopathy, with the largest lymph node measuring 5 $\times$ 17 mm. A lymph node biopsy revealed benign sinus histiocytic hyperplasia. Treatment with prednisone, 40 mg daily for 2 months, normalized his eosinophil count, but abdominal cramping persisted. Eosinophilia (eosinophil count at this time, 2,158–4,454/mm^3) rebounded after discontinuation of prednisone therapy. He was treated empirically for a parasitic infestation with mebendazole for 3 days, with partial improvement of his abdominal cramping. Hypereosinophilic syndrome was considered, and he was treated with imatinib, 100 mg daily for 5 weeks and then 400 mg daily for 8 weeks, without improvement of his eosinophilia. Eight months after his initial presentation, he was referred to us for further evaluation. A detailed history revealed that he had cut his hand while repairing a broken sewer line 1 week before the onset of his symptoms. Stool ova and parasite evaluation was repeated 3 times. All 3 samples were positive for *Strongyloides stercoralis*. He received ivermectin, 18 mg (0.2 mg/kg) daily for 5 days. A subsequent eosinophil count was normal 4 weeks after treatment. Results of repeated stool studies for ova and parasites were negative, and abdominal cramping completely resolved.

Worldwide, helminth infestations are the leading cause of eosinophilia. *Strongyloides* is found routinely in the tropics and subtropics and in certain areas of the United States, such as the southern Appalachian region, and an estimated 55 million to 100 million people are infected worldwide.[1] *Strongyloides* enters the body via the skin and then travels hematogenously to the lungs before migrating to the gastrointestinal tract.[1] The initial stool ova and parasite evaluation was performed once 2 weeks after the patient cut his hand while working on the sewer line. The rate of detection of *Strongyloides* larvae in 1 stool sample is 25% and would be a possible explanation for the initially negative stool sample result.[1–3] In addition, parasite excretion is intermittent, and, thus, it is important to obtain a minimum of 3 stool samples at least 24 hours apart.[4] Use of corticosteroids and other immunosuppressants can lead to life-threatening disseminated *Strongyloides* infestations.[5] Thus, ruling out parasitic infestation is critical before initiation of treatment with corticosteroids or other immunomodulators.

This patient illustrates the importance of a detailed history in the workup of persistent eosinophilia. Evaluation for *Strongyloides* infestation should start with stool studies for ova and parasites. If clinical suspicion remains high in a setting of negative stool studies, *Strongyloides* IgG levels should be evaluated. If suspicion remains, an endoscopic evaluation with biopsy and aspirate samples of the duodenum can be considered.[6]

Treatment for strongyloidiasis is ivermectin, with several studies[7,8] demonstrating its superiority compared with albendazole and thiabendazole. The dosing recommendation is 200 μg/kg as 1 oral dose. If disseminated disease is a concern or the patient is immunosuppressed, it is usually necessary to prolong or repeat therapy.[9]

Klion et al[10] proposed diagnostic and therapeutic algorithms for the evaluation of hypereosinophilic syndromes. Parasitic infestation can be an elusive diagnosis, and reevaluation may be necessary 4 to 6 weeks after the possible exposure. A detailed dissertation on the differential diagnosis of hypereosinophilia has recently been published.[11]

Disclosures: Dr Zuhair serves on a data and safety monitoring board for Novartis and for the National Institute of Allergy and Infectious Diseases, has an unrestricted educational grant from Talecris Biotherapeutics, and serves as a primary investigator and receives financial support from the National Institutes of Health and Veterans Affairs merit review.

In summary, parasitic infestation is not the leading cause of eosinophilia in the United States, but it can occur. Awareness of parasites and their life cycles is imperative in the evaluation of eosinophilia and may spare patients the cost and risk of testing and immunosuppressive therapy.

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IDENTIFICATION OF A NOVEL 17-kDa PROTEIN AS A FERRET ALLERGEN

Domestic ferret (*Mustela putorius furo*) is a mammal from the Mustelidae family. It is the third most common uncaged pet in North America after dogs and cats. In Europe, its popularity is progressively increasing, and it is also becoming a common pet. The role of cats and dogs as a cause of allergy is well known. However, ferrets are not so widely studied as a source of relevant allergens.

A 29-year-old man had a 3-year history of mild perennial asthma. He reported aggravation of these symptoms in the previous 2 years, coinciding with the entry of 2 ferrets into his house.

After obtaining informed consent, skin prick tests (SPTs) to common commercial aeroallergens were performed, with positive reactions to grass pollens, dust mites, molds, and epithelium from cat, dog, horse, and rabbit. Samples of ferret hair and ferret urine (obtained from the bedding material) were homogenized in phosphate-buffered saline, and, after centrifugation, dialysis, and further lyophilization, protein extracts were obtained. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)[1] after Coomassie staining showed protein bands ranging from 14 to 66 kDa in both types of ferret extracts (FEs). Ferret urine immunoblotting revealed IgE-binding proteins of 59, 34, 25, and 17 kDa in reducing conditions (with 2-mercaptoethanol), whereas in nonreducing conditions (without 2-mercaptoethanol), bands of 42, 23, 18, 16, and 15 kDa were detected (Fig 1A). The SDS-PAGE immunoblotting was also performed with extracts from dog dander; cat, horse, and rabbit epithelium; and urine from cat, rat, squirrel, and hamster. A 19-kDa IgE-binding band was revealed with extracts from dog dander, cat epithelium, and hamster urine. An enzyme allergosorbent test inhibition assay using commercial dog dander extract as solid phase showed 40% of IgE-binding inhibition when male urine FE was used as inhibitor at 10 mg/mL. The SDS-PAGE immunoblotting inhibition assays with male ferret urine as inhibitor and dog and cat epithelium extracts in solid phase showed total IgE-binding inhibition on 19- and 39-kDa bands, respectively.

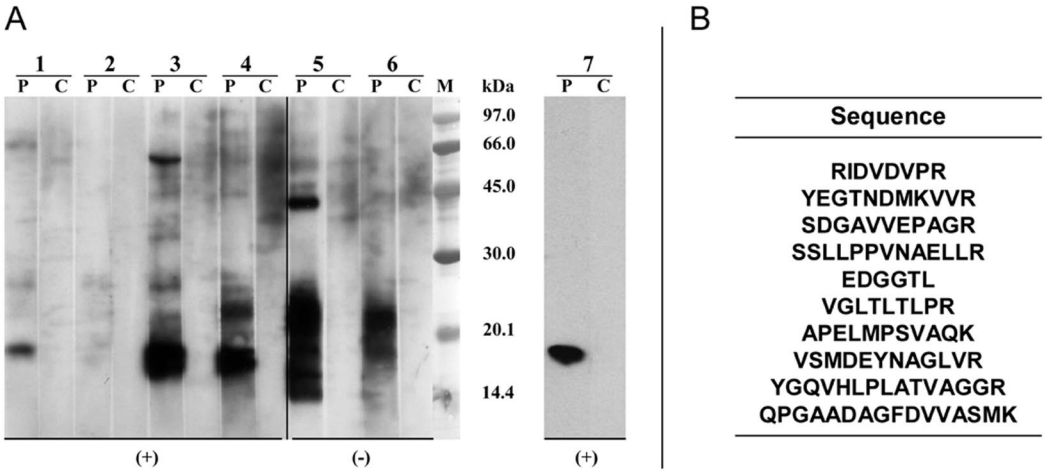

Figure 1. A, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting results. Lane 1 shows male ferret hair; lane 2, female ferret hair; lanes 3 and 5, male ferret urine; lanes 4 and 6, female ferret urine; and lane 7, 17-kDa purified protein from male ferret urine. P indicates patient serum; C, control serum (pool of sera from nonatopic subjects); M, molecular mass marker; +, sample with 2-mercaptoethanol; and −, sample without 2-mercaptoethanol. B, Peptide information for the 17-kDa protein.

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 al[3] 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 al[5] 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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Disclosures: Authors have nothing to disclose.

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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

Disclosures: Authors have nothing to disclose.

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, *Alternaria*, 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 15,000*g* 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 μg, 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 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.

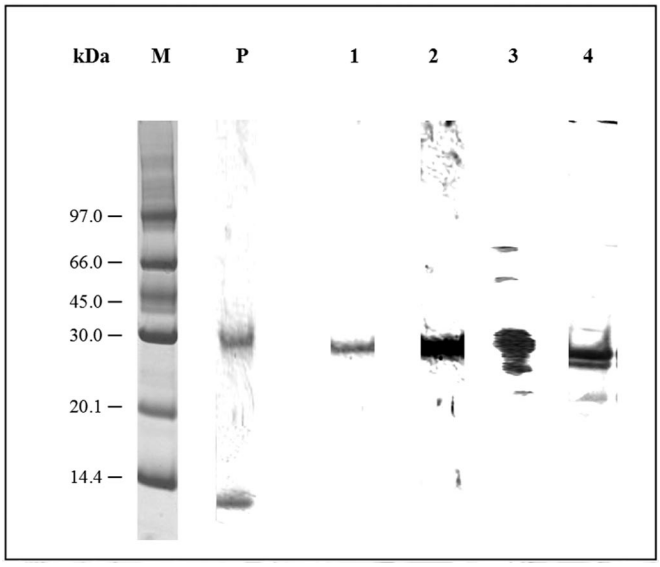

Figure 1. *Punica granatum* extract electrophoresis and immunoblotting results. Lane M indicates molecular mass markers; lane P, pomegranate extract gel electrophoresis results; and lanes 1, 2, 3, and 4, immunoblotting with serum samples from patients with a reaction after ingestion of *P granatum*.

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