

Clinical manifestations, co-sensitizations, and immunoblotting profiles of buckwheat-allergic patients

E. Heffler¹, F. Nebiolo¹, R. Asero², G. Guida¹, I. Badiu¹, S. Pizzimenti¹, C. Marchese³, S. Amato⁴, G. Mistrello⁴, F. Canaletti⁵ & G. Rolla¹

¹Allergy and Clinical Immunology, University of Torino, ASO Mauriziano "Umberto I", Torino; ²Allergy, Clinica San Carlo, Paderno Dugnano (MI); ³Clinical Pathology Laboratory, ASO Mauriziano "Umberto I", Torino; ⁴Research & Development Department, Lofarma S.p.A., Milano; ⁵Nutritional Unit, ASO Mauriziano "Umberto I", Torino, Italy

To cite this article: Heffler E, Nebiolo F, Asero R, Guida G, Badiu I, Pizzimenti S, Marchese C, Amato S, Mistrello G, Canaletti F, Rolla G. Clinical manifestations, co-sensitizations and immunoblotting profiles of buckwheat-allergic patients. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02469.x.

Keywords

anaphylaxis; buckwheat; food allergy; immunoblotting.

Correspondence

Dr. Enrico Heffler, Allergologia – Azienda Sanitaria Mauriziano "Umberto I", Largo Turati 62, 10128 Torino, Italy.

Tel.: +390115082553

Fax: +390115682558

E-mail: enrico_heffler@yahoo.it

Accepted for publication 13 July 2010

DOI:10.1111/j.1398-9995.2010.02469.x

Edited by: Reto Cramer

Abstract

Background: Buckwheat allergy is a rare food allergy in Europe and North America, whereas it is often described and studied in Asia. The aim of this study was to describe a series of patients with proven buckwheat allergy evaluated in an Italian allergy clinic. Co-sensitization to other food and inhalant allergens and immunoblotting profiles of buckwheat-allergic patients were studied.

Methods: Patients with suspected buckwheat allergy who attended the allergy clinic between January 1, 2006, and September 30, 2008, were evaluated. All patients underwent skin prick tests for a standard panel of inhalant and food allergens, prick-by-prick with buckwheat flour, buckwheat-specific IgE determinations, and double-blind placebo-controlled food challenge (DBPCFC) with buckwheat flour. Immunoblotting with buckwheat flour extract was performed on sera from buckwheat-allergic patients.

Results: Among 72 patients with suspected buckwheat allergy, 30 (41.7%) were sensitized to buckwheat and 24 had a positive DBPCFC. The mean buckwheat IgE level was 6.23 kUA/l (range, 0.16 to >100 kUA/l). Several IgE-binding proteins were identified and grouped into three patterns: a 16-kDa band in patients with predominantly gastrointestinal symptoms with grass and wheat flour co-sensitization, a 25-kDa band in patients with predominantly cutaneous symptoms and a low frequency of co-sensitization, and a 40-kDa band in patients with anaphylaxis and a low frequency of co-sensitization.

Conclusions: Buckwheat allergy is an emerging food allergy in Italy. We identified three distinct patterns of clinical and laboratory characteristics, suggesting that specific allergens could be more frequently associated with clinical manifestations of different severity.

Buckwheat (*Fagopyrum esculentum*), a member of *Polygonaceae* family, is a crop that is taxonomically unrelated to wheat and frequently used to obtain a dark flour that is the main ingredient of several Asiatic and Russian dishes. In Europe, buckwheat flour is used mostly for regional traditional dishes like 'french gallettes' (a type of crepe from Brittany), Dutch 'poffertjes' (small pancakes) or Italian 'pizzoccheri' (a kind of pasta from a small regional area, Valtellina, in northern Italy), and 'Polenta Taragna' (a hot porridge from northern Italy). Buckwheat flour is gluten-free and commonly serves as a supplement for patients with celiac disease.

Several cases of buckwheat allergy have been described, including occupational allergy in patients working in the production of buckwheat-containing foods (1, 2) and food allergies in patients who had hypersensitivity reactions after ingesting dishes containing buckwheat (3). Manifestations include skin reactions like urticaria (4), angioedema (5), and atopic dermatitis exacerbation (6); gastrointestinal involvement; respiratory symptoms like asthma (7) and Heiner syndrome (8), interstitial cystitis (9), and anaphylaxis (10–12).

Buckwheat is estimated to be the causative allergen in about 3.4% and 2.9% of all anaphylaxis in Japan (13) and

Korea (14), respectively. In France, the prevalence of buckwheat-induced food allergic reactions was about 4.5% of all food allergies recorded by the French Allergy Vigilance Network between 2002 and 2006 (15). In Italy, buckwheat allergy has rarely been reported, accounting about 1% of type I food allergies according to a recent epidemiological survey of Italian adults attending Allergy Clinics (16).

Buckwheat is also described as a possible hidden allergen in dishes that were not supposed to contain it, like wheat-burgers in one case (17), a pepper compound in another (18), and pizza in a case of anaphylaxis in a young woman we recently described (10).

Several buckwheat proteins are described as being able to bind IgE in allergic patients, and those proteins with molecular weights of 9, 16, 19, and 24 kDa are considered major allergens. The 19-kDa protein was also described as being relatively specific for buckwheat-allergic patients (19).

Cross-reactions between buckwheat and latex (*Hevea brasiliensis*) (20), rice (21), and poppy seeds (22) have been described. Most of the patient series reported are in non-European populations, who may have specific patterns of allergic sensitization.

In this study, we describe 24 patients with allergic reactions to buckwheat confirmed by double-blind placebo-controlled food challenge (DBPCFC). This case series consists mostly of patients with suspected allergy for unknown foods who received a diagnostic workup of suspected flour allergies after we became aware of buckwheat as a possible hidden allergen contained in pizza dough prepared from a mixture of wheat and buckwheat flours (10). Sensitization/allergy to other foods and/or inhalant allergens, and the buckwheat IgE-binding profile determined by SDS-PAGE/immunoblotting were investigated in all patients.

Methods

Patients

Among patients referred to the Allergy Clinic of Mauriziano Hospital in Turin (Italy) from January 1, 2006, until September 30, 2008, buckwheat allergy was suspected in 72 consecutive patients based on symptoms elicited by ingestion of foods containing buckwheat (*Polenta Taragna*, *pizzoccheri*, *french gallettes*) or foods that could potentially contain a mixture of flours (pizza, multi-grain bread, muesli bars, etc.). Twenty-four patients (19 women with a mean age of 30.6 years; range, 17–57 years) were diagnosed with buckwheat allergy and were included in the study. The diagnosis was based on clinical history, prick-by-prick with buckwheat flour, specific buckwheat IgE, and positive DBPCFC with buckwheat flour. According to the major clinical manifestations reported in their clinical histories and recorded during DBPCFC, patients were classified into three groups: isolated cutaneous symptoms (urticaria and/or angioedema, or exacerbations of atopic dermatitis), prevalent gastrointestinal symptoms (nausea/vomiting, and/or abdominal pain with diarrhea, with or without mild cutaneous symptoms, erythema with few wheals), or anaphylaxis diagnosed according to the

involvement of two or more organ systems (skin, respiratory, gastrointestinal, and cardiovascular) (23).

All patients provided informed consent for DBPCFC, and the study was approved by the Institutional Ethics Review Board.

Skin prick tests

All patients underwent skin prick tests for a standard panel of 12 inhalant allergens (*Betula verucosa*, *Corylus avellana*, *Lolium spp*, *Parietaria judaica*, *Ambrosia artemisiifolia*, *Artemisia vulgaris*, *Dermatophagoides pteroyssinus*, *Alternaria alternata*, *Aspergillus fumigatus*, and cat and dog dander), 10 food allergens (peanuts, walnuts, hazelnuts, soybeans, peaches, wheat, rice, corn, hen's eggs, and cow's milk), and latex (*H. brasiliensis*). All patients also underwent prick-by-prick with buckwheat flour (diluted 1 : 1 in saline). To exclude non-specific irritant reactions, prick-by-prick with buckwheat flour was also tested in 10 atopic and 10 non-atopic control subjects who did not exhibit any reaction. Histamine and saline served as positive and negative control solutions, respectively. A wheal that was 3 mm larger than the negative control was considered a positive skin-prick test.

Determination of allergen-specific IgE antibodies

IgE-specific antibodies for buckwheat were determined in all patients according to the manufacturer's instructions (Phadia, Uppsala, Sweden). The results were expressed in kUA/l, and a positive value was considered one greater than 0.10.

Double-blind placebo-controlled food challenge

All patients sensitized to buckwheat underwent the DBPCFC for buckwheat flour.

The patients were given test meals in our Hospital Allergy Clinic on separate days after an overnight fast and withdrawal of all anti-allergy drugs. The physician and patient were both blinded to the protocol. The test meal was prepared by dieticians at the Mauriziano Hospital. The ingredients used to prepare the active meal were raw buckwheat flour, potato starch, bitter cocoa powder, sugar, water, and peppermint oil. The same ingredients were used for the placebo meal apart from the addition of buckwheat flour, which was replaced by an equivalent amount of potato starch instead. A DBPCFC with up to 25 g of buckwheat flour was performed in all study patients. Precautions were taken for possible systemic reactions. Intravenous access was obtained for all the patients. Before administering the test meal, the skin was carefully inspected for pre-existing lesions, and blood pressure and forced expiratory volume in 1 s (FEV₁) were measured in all patients. Spirometry was performed before each subsequent dose if there was a history of asthma and whenever respiratory symptoms were reported during the challenge. A positive reaction for asthma was defined as a decrease in FEV₁ of at least 15% compared with baseline. Active and placebo test meals were administered on separate days under the supervision of one trained nurse and one

physician. Doses were delivered at 20-min intervals. A minimum starting dose of 100 mg of raw buckwheat flour was administered; subsequently, the following doses were administered (cumulative dose is shown in parentheses): 500 mg (600 mg), 1 g (1.6 g), 1.5 g (3.1 g), 3.5 g (6.6 g), 6.4 g (13 g), and 12 g (25 g). The total cumulative dose administered was 25 g of raw buckwheat flour. All doses were administered unless convincing symptoms appeared. As indicated by the European Academy of Allergy and Clinical Immunology position paper (24), patients with negative DBPCFC underwent open challenge with 25 g of cooked buckwheat flour (*Polenta Taragna*).

Allergen extract

Raw buckwheat flour was defatted with ethyl ether, then extracted with 20% (w/v) 1.5 M NaCl containing 0.05% NaN₃ for 2 h at room temperature. The extract was centrifuged at 18 000 g for 30 min at 4°C to remove the precipitate. The supernatant was dialyzed (cutoff molecular weight of 3.5 kDa; Spectrum, Prodotti Gianni, Milan, Italy) against distilled water for 48 h. The protein concentration of this extract, determined by the Lowry method (25), was 8.32 mg/ml. The extract was then concentrated using membrane ultrafiltration with Amicon cells (Amicon Inc, Beverly Millipore Corp, Bedford, MA, USA) with a molecular weight cutoff of 3500 Da to reach a protein concentration of approximately 12 mg/ml.

Identification of buckwheat allergens; SDS-PAGE immunoblotting

In all patients with proven buckwheat allergy, the IgE-binding profile of buckwheat was determined using SDS-PAGE/immunoblotting. Briefly, the serum sample was diluted 1 : 2 with SDS sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan, Italy) and 5% beta-mercaptoethanol. Electrophoresis was run at 180 mA for 1 h in a 10% polyacrylamide precast gel (Nupage Bis-Tris, Novex, Prodotti Gianni). The separated proteins were transferred onto a nitrocellulose membrane (0.4 mm; Amersham, Buckinghamshire, UK) according to Towbin et al. (26), saturated with 0.1 mol/L tris-buffered saline containing 5% fat-free milk powder, and incubated for 16 h at 4°C with each patient's serum. After washing, a second incubation was performed with peroxidase-conjugated anti-human IgE antibodies (Biospecific, Emeryville, CA, USA) diluted 1 : 3500 in saturation buffer using an ECL western blotting kit (Amersham, Milan, Italy).

The results of the immunoblotting analysis were evaluated by a reader blinded to the patient's allergy results.

Statistical analysis

Descriptive analyses were performed. Continuous variables were evaluated with the normality test of Kolmogorov-Smirnov and compared with ANOVA or the Mann-Whitney test, depending on the normality of distribution. Categorical variables were compared using Fisher's exact test.

All data were analyzed using SPSS v. 16.0 software (SPSS, Chicago, IL, USA).

Results

Patient selection and clinical manifestations of buckwheat allergy

Among 72 patients with suspected buckwheat allergy, 30 (41.7%) exhibited sensitization to buckwheat (by means of prick-by-prick and/or specific IgE). Twenty-four of the 30 patients sensitized to buckwheat had a positive DBPCFC and were included in the study. The mean age of enrolled patients was 30.6 years (range, 17–57 years), 19 (79.2%) were women, and 16 (66.7%) had a previous diagnosis of allergic rhinitis, which was associated with asthma in 6 (37.5%) patients. The clinical and demographic characteristics of the patients are listed in Table 1.

Reported allergic manifestations in the patients' clinical history were cutaneous (urticaria, angioedema, exacerbation of atopic dermatitis) in eight patients (33.3%), gastrointestinal (nausea, vomiting, abdominal pain with diarrhea, which was associated with mild cutaneous involvement in three patients) in 7 (29.2%), and anaphylaxis in 7 (29.2%). Anaphylaxis consisted of the combination of hypotension, generalized urticaria, and abdominal pain in four patients; laryngeal oedema, generalized urticaria, dyspnea, and dysphagia in one patient; and generalized urticaria, facial angioedema, abdominal pain, diarrhea, and nausea and vomiting in two patients. Two patients (8.3%) reported respiratory symptoms only, which consisted of dyspnea with wheezing and severe nasal obstruction.

It is noteworthy that buckwheat was the main ingredient of the ingested food that caused the allergic reaction in only three cases, whereas in the remaining 21 cases, buckwheat was a hidden allergen in dishes that were not supposed to contain it, like pizza, in 13 patients (Table 1).

Double-blind placebo-controlled food challenge

All the test meals were well accepted by all patients, and no severe reaction was manifested during any of the challenges. All patients with positive challenge tests developed symptoms either during the challenge or immediately after test completion. The symptoms, which were documented during the positive buckwheat DBPCFCs, were consistent with those reported in the patient's clinical history and are reported together with provocation doses in Table 2. The mean provocation dose was 11.4 ± 2.0 g.

Clinical manifestations occurring during DBPCFC were treated with oral anti-histamines in all 24 patients, salbutamol MDI in two patients; i.m. 20 mg joscine *N*-butylbromide for relief of abdominal pain in five patients; and i.v. saline, oxygen supplementation, i.v. 40 mg prednisolone, i.m. 0.3 ml epinephrine, and a 6-hour observation period in four cases of anaphylaxis (patients 4, 5, 13, and 24 in Table 2), who recovered completely. No patient required hospitalization.

Table 1 Demographic, clinical and laboratory characteristics of patients with buckwheat allergy

ID	Gender	Age	Clinical involvement	Involved food	Specific IgE (kUA/l)	Other sensitizations										Immunoblotting analysis					
						Bet	Phl	Amb	DP/F	Ara	Tri	Gly	Jug	Pru	Hev	10 kDa	16 kDa	25 kDa	30 kDa	40 kDa	67 kDa
1	F	29	Gastrointestinal	Pizza	5.12	+	+	+	+	+	+	+				+	+				+
2	F	36	Gastrointestinal	French gallettes	1.51	+	+	+	+		+	+				+	+				+
3	M	24	Respiratory	Pizza	0.52	+	+	+	+	+	+	+	+						+		+
4	F	57	Anaphylaxis	Pizzoccheri	4.09										+	+	+	+			
5	F	33	Anaphylaxis	Pizza	10.40							+									+
6	M	19	Skin	Multicereal bread	4.97					+		+	+	+		+	+				+
7	F	44	Gastrointestinal	Pizza	0.50	+	+	+		+	+	+				+	+				
8	F	22	Anaphylaxis	Pizza	0.16	+	+	+		+	+	+		+						+	
9	F	29	Skin	Multicereal pasta	5.20	+	+		+	+	+	+	+				+				+
10	F	19	Anaphylaxis	Pizza	1.02	+	+	+		+	+	+	+	+							+
11	F	32	Skin	Multicereal bread	0.18	+	+	+	+	+	+	+	+	+			+				+
12	F	26	Skin	Pizza	5.57			+		+	+	+	+						+		
13	F	31	Anaphylaxis	Cake	3.23					+	+	+		+					+		
14	M	39	Gastrointestinal	Pizza	0.28	+	+	+	+	+	+	+									
15	M	28	Gastrointestinal	Pizza	1.11	+	+	+	+	+	+	+	+				+				+
16	F	18	Skin	Muesli bar	0.32	+	+		+	+	+	+				+	+				+
17	M	24	Gastrointestinal	Pizza	0.49	+	+		+	+	+	+	+	+		+	+				
18	F	21	Gastrointestinal	Pizza	1.32	+	+	+			+	+				+	+				
19	F	48	Respiratory	Multicereal pasta	0.32										+						+
20	F	41	Skin	Multicereal bread	0.19					+		+				+	+			+	
21	F	34	Skin	Pizza	0.36	+		+		+		+	+	+		+	+	+		+	
22	F	19	Anaphylaxis	Multicereal snack	0.22							+		+						+	
23	F	17	Skin	Pizza	2.57	+		+				+									
24	F	45	Anaphylaxis	Polenta Taragna	>100				+		+	+	+			+	+		+		+

Bet, *Betula alba* (Birch pollen); Phl, *Phleum pratense* (Grass pollen); Amb, *Ambrosia artemisiifolia* (Ambrosia pollen); DP/F, *Dermatophagoides Farinae/Pteronissynus* (House Dust Mites); Ara, *Arachys hypogaea* (Peanut); Tri, *Triticum* spp. (Wheat); Gly, *Glycine max* (Soybean); Jug, *Juglans regia* (Walnut); Pru, *Prunus persica* (Peach); Hev, *Hevea brasiliensis* (Latex).

Table 2 Positive double-blind placebo-controlled food challenge results

ID	DBPCFC		Clinical involvement	PD (g)	ID	DBPCFC		Clinical involvement	PD (g)
	A	P				A	P		
1	+	–	AP, D, E, Pr	3.1	13	+	–	AN (H, U, AP)	0.6
2	+	–	N, V	13.0	14	+	–	N, V, AP, Pr	1.6
3	+	–	DPN, W, NO	25.0	15	+	–	AP, D	25.0
4	+	–	AN (H, U, AP)	25.0	16	+	–	U, AE	25.0
5	+	–	AN (H, U, AP)	0.6	17	+	–	N, V, U	6.6
6	+	–	U, AE	6.6	18	+	–	N, V, AP, D	13.0
7	+	–	N, V	25.0	19	+	–	DPN, W, NO	25.0
8	+	–	AN (U, AE, DPG, DPN)	13.0	20	+	–	U	13.0
9	+	–	U	1.6	21	+	–	U, AE	3.1
10	+	–	AN (U, AE, AP, D, N, V)	1.6	22	+	–	AN (U, AE, AP, D, N, V)	13.0
11	+	–	AD, Pr	25	23	+	–	AD, E, Pr	1.6
12	+	–	U	6.6	24	+	–	AN (H, U, AP)	0.6

DBPCFC, double-blind placebo-controlled food challenge; A, active meal; P, placebo meal; PD, provocation dose; AD, atopic dermatitis flare; AE, angioedema; AN, anaphylaxis; AP, abdominal pain; D, diarrhea; DPG, dysphagia; DPN, dyspnea; E, erythema; H, hypotension; N, nausea; NO, nasal obstruction; Pr, pruritus; U, urticaria; V, vomiting; W, wheezing.

Skin prick tests

The vast majority of patients had multiple food allergen sensitizations (soybean, 20/24, 83.5%; peanuts, 16/24, 66.7%; wheat, 14/24, 58.3%; walnut, 8/24, 33.3%; peach, 6/24, 25.0%), as well sensitization to inhaled allergens (grass pollen, 13/24, 54.2%; weeds, *Artemisia absinthium* and *Ambrosia artemisiifolia*, 13/24, 54.2%; birch, 8/24, 37.5%; and house dust mites, 8/24, 37.5%). Only two patients were monosensitized to buckwheat, and one patient was also sensitized to latex.

IgE and immunoblotting

The mean specific IgE value for buckwheat was 6.23 kUA/l, ranging from 0.16 to >100 kUA/l. There was no statistically significant difference in mean specific IgE levels in patients reporting cutaneous symptoms (2.42 ± 0.87 kUA/l), gastro-

intestinal manifestations (1.47 ± 0.63 kUA/l), or anaphylaxis (17.0 ± 13.9 kUA/l). Nevertheless, the two patients with the most elevated specific IgE reported anaphylaxis (Table 1).

The SDS-PAGE results for buckwheat extract showed different proteins with apparent molecular weights ranging from 10 to 67 kDa. Buckwheat IgE immunoblotting profiles for each single patient are reported in Table 1 (also see Fig. 1). Twelve of 24 patients (50%) had specific IgE against a protein band at 67 kDa. Other allergens were detected at 16 kDa (12 patients; 50%), 25 kDa (six patients; 25%), 30 kDa (six patients; 25%), 40 kDa (five patients; 20.8%), and 10 kDa (five patients; 20.8%). Sera from patients 4 and 19, who were monosensitized to buckwheat, recognized the 10-, 16-, 25-, and 30-kDa proteins and the 10- and 67-kDa proteins, respectively.

All patients with predominant gastrointestinal symptoms were sensitized to wheat and grass pollen and recognized the 16-kDa allergen more often than the other two groups (6/7

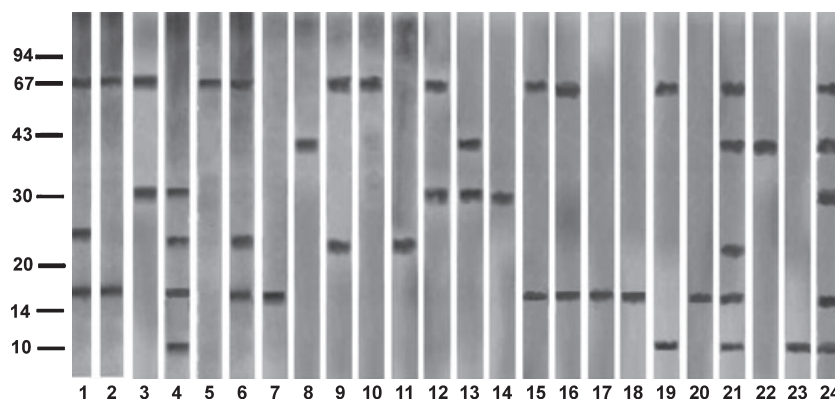


Figure 1 IgE immunoblotting of buckwheat extract in sera from 24 patients with positive double-blind placebo-controlled food challenge results.

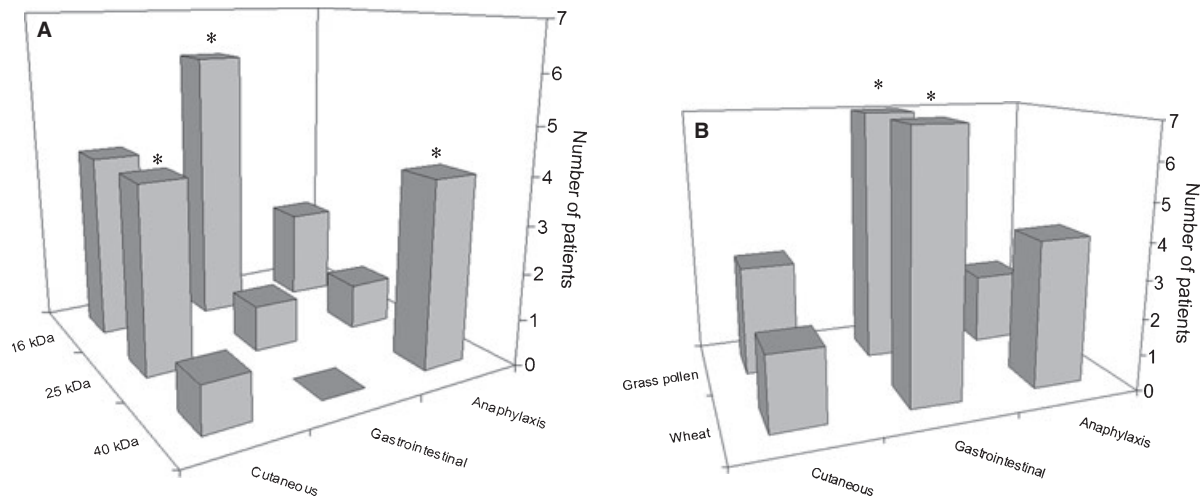


Figure 2 Distribution of buckwheat IgE-binding proteins (Panel A) and main co-sensitizations (Panel B) in patients with buckwheat allergy according to different clinical manifestations. * $P \leq 0.05$.

patients, 85.7%, compared with 4/8 [50%] in the cutaneous group and 2/7 [28.6%] in the anaphylaxis group; $P = 0.03$). Patients reporting anaphylaxis had IgE that recognized the 40-kDa protein more frequently than patients in the other two groups (4/7 [57.1%] compared with 1/8 [12.5%] patients with cutaneous symptoms, and none of those with gastrointestinal or respiratory symptoms; $P = 0.01$). The 25-kDa protein was more frequently recognized by IgE from patients reporting cutaneous symptoms (4/6 [66.7%] compared with 1/7 [14.3%] in the gastrointestinal and 1/7 [14.3%] in the anaphylaxis groups; $P = 0.05$) (Fig. 2).

Discussion

This is, to our knowledge, the largest case series of buckwheat allergy reported outside of Asia. In this study, we describe 24 consecutive patients with proven food allergy to buckwheat, representing 7.3% of all newly diagnosed food allergy patients we observed during the same study period (33 months, *data not shown*). It is remarkable that the vast majority of our patients experienced food allergy to buckwheat as a hidden allergen in common Italian foods such as pizza or pasta. The prevalence of buckwheat-induced allergy we report is higher than that reported in the Italian EpidemAAITO study (16). In this study, the authors underlined that buckwheat is typically used in limited, Alpine areas of the country but virtually unknown in Southern Italy. We believe another explanation for our results is the increasing use of buckwheat flour as an ingredient in pizza dough in Northern Italy (10). The prevalence of buckwheat allergy we found in this study is similar to that reported by the French Allergy Vigilance Network (15). In France, several dishes commonly eaten by a large proportion of the population (such as french gallettes) are made with buckwheat flour, increasing the risk of sensitization. Our study shows that in buckwheat DBPCFCs symptoms were

induced at a dose ≤ 1.6 g in 7/24 patients, a percentage quite similar to that observed for wheat-allergic patients (27).

The immunoblotting analysis showed that there were six buckwheat-specific IgE-binding bands in our patients, most of which probably correspond to previously identified buckwheat allergens; the 10-kDa Fag e protein (28) and the Fag e 16-kDa protein (29), both members of the 2S-albumins family that are structurally homologous to other 2S-albumins-like allergens in peanuts, soybeans, or walnuts; the Fag e 1 24-kDa protein (19) or the Fag e 19-kDa proteins (30), both members of the cupins family; and the 40- and 67-kDa allergen bands previously described by Nair et al. (31).

The different clinical manifestations of buckwheat allergy reported by our patients seem to be associated with characteristic patterns of pollen and foods co-sensitization, and immunoblotting profiles. This observation indicates that some allergens may be able to elicit reactions that are more severe than others, as reported for fruit allergy where lipid transfer protein sensitization is responsible for the more severe reactions (32). Further studies must be performed to better characterize buckwheat allergens and to understand the mechanisms involved in the generation of different clinical manifestations in allergic patients.

In conclusion, this study shows that, at least in Northern Italy, buckwheat is an emerging and intriguing allergenic source, often a hidden allergen, which can cause systemic reactions ranging from isolated cutaneous manifestations to anaphylaxis in 30% of cases. We recommend that clinicians suspect and test for buckwheat allergy during the evaluation of patients presenting with systemic reactions suggestive of food allergy.

Conflicts of Interest

No conflicts of interest to declare.

References

- Park HS, Nahm DH. Buckwheat flour hypersensitivity: an occupational asthma in a noodle maker. *Clin Exp Allergy* 1996;**26**:423–427.
- Valdivieso R, Moneo I, Pola J, Munoz T, Zapata C, Hinojosa M et al. Occupational asthma and contact urticaria caused by buckwheat flour. *Ann Allergy* 1989;**63**:149–152.
- Wieslander G, Norback D. Buckwheat allergy. *Allergy* 2001;**56**:703–704.
- Smith HL. Buckwheat-poisoning with report of a case in man (1909). *Allergy Proc* 1990;**11**:193–196.
- Bourrier T, Noormahomed T, Coache M, Albertini M, Boutte P. A case of buckwheat allergy in a child. *Revue Francaise d'allergologie et d'immunologie clinique* 2003;**43**:530–532.
- Chandrupatla CV, Kundu RV, Aronson IK. Buckwheat allergy and atopic dermatitis. *J Am Acad Dermatol* 2005;**53**:356–357.
- Stember RH. Buckwheat allergy. *Allergy Asthma Proc* 2006;**27**:393–395.
- Agata H, Kondo N, Fukutomi O, Takemura M, Tashita H, Kobayashi Y et al. Pulmonary hemosiderosis with hypersensitivity to buckwheat. *Ann Allergy Asthma Immunol* 1997;**78**:233–237.
- Ogawa H, Nakamura Y, Tokinaga K, Sakakura N, Yamashita M. [Case of interstitial cystitis accompanied by food allergy]. *Arerugi* 2005;**54**:641–645.
- Heffler E, Guida G, Badiu I, Nebiolo F, Rolla G. Anaphylaxis after eating Italian pizza containing buckwheat as the hidden food allergen. *J Invest Allergol Clin Immunol* 2007;**17**:261–263.
- Schiffner R, Przybilla B, Burgdorf T, Landthaler M, Stolz W. Anaphylaxis to buckwheat. *Allergy* 2001;**56**:1020–1021.
- Wang TC, Shyr SD, Wen DC, Kao YH, Huang LH. Buckwheat anaphylaxis: an unusual allergen in Taiwan. *Asian Pac J Allergy Immunol* 2006;**24**:167–170.
- Imamura T, Kanagawa Y, Ebisawa M. A survey of patients with self-reported severe food allergies in Japan. *Pediatr Allergy Immunol* 2008;**19**:270–274.
- Yang MS, Lee SH, Kim TW, Kwon JW, Lee SM, Kim SH et al. Epidemiologic and clinical features of anaphylaxis in Korea. *Ann Allergy Asthma Immunol* 2008;**100**:31–36.
- Beaudoin E, Sergeant P, Flabbee J, Morisset M, Kanny G, Moneret-Vautrin DA. Buckwheat allergy: analysis of 22 cases recorded by the allergy vigilance network (2002–2006). *Eur Ann Allergy Clin Immunol* 2007;**39**:303–306.
- Asero R, Antonicelli L, Arena A, Bommarioto L, Caruso B, Crivellaro M et al. Epidem-AAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin Exp Allergy* 2009;**39**:547–555.
- Wuthrich B, Trojan A. Wheatburger anaphylaxis due to hidden buckwheat. *Clin Exp Allergy* 1995;**25**:1263.
- Yuge M, Niimi Y, Kawana S. A case of anaphylaxis caused by buck wheat as an addition contained in pepper. *Arerugi* 2001;**50**:555–557.
- Park JW, Kang DB, Kim CW, Koh SH, Yum HY, Kim KE et al. Identification and characterization of the major allergens of buckwheat. *Allergy* 2000;**55**:1035–1041.
- Maat-Bleeker F, Stapel SO. Cross-reactivity between buckwheat and latex. *Allergy* 1998;**53**:538–539.
- Yamada K, Urisu A, Morita Y, Kondo Y, Wada E, Komada H et al. Immediate hypersensitive reactions to buckwheat ingestion and cross allergenicity between buckwheat and rice antigens in subjects with high levels of IgE antibodies to buckwheat. *Ann Allergy Asthma Immunol* 1995;**75**:56–61.
- Oppel T, Thomas P, Wollenberg A. Cross-sensitization between poppy seed and buckwheat in a food-allergic patient with poppy seed anaphylaxis. *Int Arch Allergy Immunol* 2006;**140**:170–173.
- Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A et al. Second symposium on the definition and management of anaphylaxis: summary report -Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;**117**:391–397.
- Bindeslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J et al. Standardization of food challenges in patients with immediate reaction to foods – position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004;**59**:690–697.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;**193**:265–275.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;**76**:4350–4354.
- Scibilia J, Pastorello EA, Zisa G, Ottolenghi A, Ballmer-Weber B, Pravettoni V et al. Maize food allergy: a double-blind placebo-controlled study. *Clin Exp Allergy* 2008;**38**:1943–1949.
- Matsumoto R, Fujino K, Nagata Y, Hashiguchi S, Ito Y, Aihara Y et al. Molecular characterization of a 10-kDa buckwheat molecule reactive to allergic patients' IgE. *Allergy* 2004;**59**:533–538.
- Satoh R, Koyano S, Takagi K, Nakamura R, Teshima R, Sawada J. Immunological characterization and mutational analysis of the recombinant protein BWp16, a major allergen in buckwheat. *Biol Pharm Bull* 2008;**31**:1079–1085.
- Choi SY, Sohn JH, Lee YW, Lee EK, Hong CS, Park JW. Characterization of buckwheat 19-kD allergen and its application for diagnosing clinical reactivity. *Int Arch Allergy Immunol* 2007;**144**:267–274.
- Nair A, Adachi T. Immunodetection and characterization of allergenic proteins in common buckwheat (*Fagopyrum esculentum*). *Plant Biotechnol* 1999;**16**:219–224.
- Pastorello EA, Vieths S, Pravettoni V, Farioli L, Trambaioli C, Fortunato D et al. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002;**109**:563–570.