

Original article

Mustard allergy confirmed by double-blind placebo-controlled food challenges: clinical features and cross-reactivity with mugwort pollen and plant-derived foods

Background: Mustard IgE-mediated allergy is supposed to be a rare cause of food allergy, and its clinical features and cross-reactivities have not been fully elucidated.

Methods: A prospective study was carried out, recruiting mustard allergic patients, and paired control subjects. A clinical questionnaire was administered, and skin-prick tests (SPT) with panels of aeroallergens and foods, serum extraction for *in vitro* tests and double-blind placebo-controlled food challenges (DBPCFC) were performed.

Results: Thirty-eight mainly adult patients, with 10.5% reporting systemic anaphylaxis, were included in the study [age (mean $\pm$ SD): 21.9 ± 8.6 years]. DBPCFC were performed in 24 patients, being positive in 14 cases (58.3%). Patients with positive outcome showed significantly greater mustard SPT than those with negative outcome (8.2 ± 3.7 vs 5.3 ± 2.4 mm, $P < 0.05$), and the receiver-operating characteristic (ROC) curve analysis yielded a cut-off value for mustard commercial SPT of 8 mm, with a specificity of 90% (95% CI, 55.5–98.3), and a sensitivity of 50% (95% CI, 23.1–76.9). A significant association between mustard hypersensitivity and mugwort pollen sensitization was found (97.4% of patients), with partial cross-reactivity demonstrated by UniCAP System inhibition assays. All patients showed sensitization to other members of Brassicaceae family, and cross-reactivity among them was also confirmed. Moreover, significant associations with nut (97.4%), leguminous (94.7%), corn (78.9%), and Rosaceae fruit (89.5%) sensitizations were also shown. Around 40% of these food sensitizations were symptomatic, including food-dependent exercise-induced anaphylaxis in six patients.

Conclusions: Mustard allergy is a not-uncommon disorder that can induce severe reactions. Significant associations with mugwort pollinosis and several plant-derived food allergies are demonstrated, suggesting a new mustard–mugwort allergy syndrome. A relationship between this syndrome and food-dependent exercise-induced anaphylaxis is also reported.

**J. Figueroa, C. Blanco,
A. G. Dumpiérrez, L. Almeida,
N. Ortega, R. Castillo, L. Navarro,
E. Pérez, M. D. Gallego, T. Carrillo**

Hospital de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Las Palmas, Spain

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Dr Carlos Blanco
Servicio de Alergia
Hospital de Gran Canaria Dr. Negrín
C/Bco. de la Ballena s/n
35020 Las Palmas de G.C.
Spain

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Mustard plants belong to the Brassicaceae family, together with broccoli, brussel sprouts, turnip, cabbage, cauliflower, kohlrabi, rutabaga, watercress, radish and rapeseed. Down the centuries, mustard has been used for culinary purposes, mainly in sauces, as a seasoning in main dishes, or as an ingredient in salad dressings. There are two major types of mustard seeds: white (*Sinapis alba*

or yellow mustard) and brown (*Brassica juncea* or oriental mustard). White mustard seeds are much larger than the brown variety but a lot less pungent, being the main ingredient in American-style mustards. White and brown seeds are blended to make English mustard. Brown mustard seeds are the main ingredient in European and Chinese mustards. Mustard seeds are sold whole, ground into powder or processed further into prepared mustard. In Spain, mustard consumption is mainly related to sauces used in fast-food restaurants.

Despite its widespread use, mustard is supposed to be a rare cause of food allergy, both in adults and children, although it has been included in a list of 12 potential

Abbreviations: CI, confidence interval; DBPCFC, double-blind placebo-controlled food challenge; MW, molecular weight; OAS, oral allergy syndrome; ROC, receiver-operating characteristic; SBPCFC, single-blind placebo-controlled food challenge; SD, standard deviation; SPT, skin-prick test.

allergenic ingredients to be labelled in a recent European Union directive on food labelling. In fact, only a few case reports of anaphylaxis to mustard in adult patients are available in the literature (1–6). Sometimes, it presents as a hidden allergen, which makes diagnosis more difficult (1, 6). Surprisingly, in one study performed in France among children, mustard allergy was ranked as the fourth aetiological agent in prevalence of food allergens – after eggs, peanuts and cow's milk (7). The same investigators have subsequently studied 36 children with positive mustard skin-prick test (SPT), 15 of them being allergic – showing positive single-blind placebo-controlled food challenges (SBPCFC) – to conclude that mustard allergy frequently starts early in life, and that clinical symptoms of mustard allergy in children seem to be not as severe as in adults (8). Moreover, clinical characteristics of 29 mustard-allergic adult patients have been reported, including systemic anaphylactic reactions in almost 50% of them (9). Recently, 24 double-blind and six single-blind placebo-controlled food challenges have been carried out in 28 children and two adults sensitized to mustard, showing that 23.3% of them were truly allergic, as defined by a positive challenge (10).

Characteristics of antigens responsible for mustard immediate hypersensitivity have been investigated by various authors. A major allergen of white mustard, Sin a 1, has been characterized as a seed storage protein, belonging to the 2S albumin family, with a molecular weight (MW) of 14 kDa (11). Its structure consists of two polypeptide chains linked by a disulphide bridge, which resists both heating and digestion by trypsin (12, 13). A major allergen of the oriental mustard, Bra j 1, has been also identified as a seed-storage protein from the 2S albumin family, with a MW of approximately 16 kDa, being closely related in structure to Sin a 1 (14, 15).

The objective of this paper is to study a group of 38 subjects with mustard IgE-mediated hypersensitivity, including double-blind placebo-controlled food challenges (DBPCFC) in 24 of them.

Methods

Subjects

A prospective study was designed in our Allergy Section, which was attended by both children and adults, and was approved by our hospital Institutional Review Board. It comprised a detailed clinical history, *in vivo* skin tests, and serum extraction for *in vitro* assays. During a 2-year period, every patient reporting immediate adverse reactions related to mustard ingestion, suggestive of being IgE-mediated, and showing positive SPT to a mustard extract, was recruited. Food immediate hypersensitivity was diagnosed on the basis of a suggestive clinical history, complemented with a positive SPT to the corresponding food. When dealing with a mustard not-severe adverse reaction, food allergy was further confirmed by DBPCFC tests. A control group of dust-mite allergic subjects, paired for age and sex with our patients, was also recruited. Written

informed consent was obtained from both patients and controls before admission in the study.

Skin-prick tests

Skin-prick tests were performed in all patients and controls according to standard procedures with a panel of commercial aeroallergen extracts including dust mites – *Dermatophagoides pteronyssinus*, *D. farinae*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor*; fungi – *Aspergillus fumigatus*, *Cladosporium herbarum* and *Alternaria alternata*; pollens – *Olea europea*, *Lolium perenne* and *Artemisia vulgaris*; animal epithelia – cat, dog, rabbit and horse; and latex (ALK-Abello, Madrid, Spain). A panel of other commercial pollen extracts were also tested, including nine grass, nine weed and nine tree pollens (ALK-Abello, Bial-Aristegui, Bilbao, Spain and CBF-Leti, Madrid, Spain).

In the same way, they were also skin tested with a panel of commercial food extracts, including milk, egg, hazelnut, peanut, banana, peach, avocado, walnut, apple, wheat, corn, and barley (ALK-Abello); chestnut, sunflower seed, kiwi, chickpea, lentil, mustard (*S. alba*) and squid (Bial-Aristegui); almond, green pea, fish and lobster (Allergy Therapeutics, Barcelona, Spain); and soy meal (CBF-Leti). Meanwhile, SPT with celery, carrot, pepper, cabbage and cauliflower were made by the prick-prick method (16), which was also used for testing patients who underwent DBPCFC with the mustard sauce, used for that purpose. Results of SPT are expressed as the mean wheal diameter (in mm) after 15 min of puncture. Histamine dihydrochloride (10 mg/ml) and physiologic saline solutions served as positive and negative controls, respectively. A mean wheal diameter > 3 mm, compared with the saline control, was considered a positive response.

Food challenges

Patients were challenged in a double-blind, placebo-controlled fashion, as described elsewhere (17). Food challenge was not performed when a patient had a history of severe anaphylaxis to mustard (adverse reaction involving at least three target organs or with demonstrated vascular collapse). A commercial yellow mustard sauce was masked in a natural yoghurt-based vehicle, containing a mix of vanilla and lemon juices, sugar and yellow colouring. Mustard sauce was composed of water, *S. alba* seeds (14% w/v), vinegar, salt, turmeric, paprika and cloves, and it was free of sulphites. Apart from mustard, all ingredients of both mustard sauce and vehicle were known to be tolerated by all patients. A previous study in mustard-non-allergic volunteers was carried out in order to demonstrate that 10 g of mustard sauce could be masked by this method, and no tingling sensation that could be mistaken as an oral allergy syndrome (OAS) was observed. Preparation of challenges was performed in the allergy laboratory in the same morning, and subjects were then randomly assigned to either food or placebo (vehicle), with a 2-h interval between the first and second part of the challenge. Increasing doses (80, 240, 800, 2400 and 6480 mg) were administered with a 15-min interval until symptoms appeared or a cumulative dose of 10 g of mustard sauce was reached. Mustard allergy was accepted if the subject had symptoms after challenge with active substance and not with placebo. An open feeding of up to 25 g of mustard sauce followed negative blinded challenges when patients wished to eat mustard.

In vitro tests

Total IgE and specific IgE to mugwort pollen, mustard, cabbage, cauliflower, broccoli, and other foods if involved in adverse

reactions, were determined in patients and controls by the UniCAP System (Pharmacia Diagnostics, Uppsala, Sweden), following the manufacturer's instructions. A specific IgE >0.35 kU/l was considered positive.

A pool of sera from five mustard-allergic patients, each showing specific IgE values to both mustard and mugwort pollen higher than 3.5 kU/l (CAP class III and more), was used for CAP inhibition assays. Duplicated samples of 50 μ l of the undiluted pool of sera were premixed with 50 μ l of five increasing 1 : 10 dilutions of the inhibitor solution in phosphate-buffered saline (PBS), pH 7.4, containing 0.03% (w/v) of serum albumin. After 1-h of incubation at room temperature, specific IgE levels to mustard and mugwort pollen were determined, following the UniCAP System manual. Commercial extracts of mustard (Bial-Aristegui), *Artemisia vulgaris* (ALK-Abello), and *C. herbarum* (ALK-Abello) – as a negative control – were used as inhibitors. Similarly, inhibition assays with each of the five sera and the same inhibitor extracts, but this time undiluted, were performed.

In the same way, sera from 11 of the 38 patients, who not only showed specific IgE values to cabbage, cauliflower and broccoli, higher than 3.50 kU/l, but also referred symptoms after ingestion of these vegetables, were used for individual CAP inhibition assays. For that purpose, duplicated 50- μ l serum samples were premixed with 50 μ l of the same inhibitor extracts mentioned above, and the specific IgE values to cabbage, cauliflower and broccoli were then determined.

Statistical evaluation

Statistical significance was tested with the Student's *t*-test or the chi-square test, as appropriate. A *P*-value of <0.05 was considered to indicate a statistically significant difference between groups. The area under the receiver operating characteristic (ROC) curve, sensitivity, and specificity were also calculated, when needed.

Results

General characteristics

Thirty-eight mustard-hypersensitive patients were included in the study, whose general characteristics are summarized in Table 1. Mean age ($\pm$ SD) was 21.9 ± 8.6 years (range 3–39), with only five patients under 14 years of age, and a slight predominance of females (20 : 18). Thirty-five patients (92.1%) were atopic, as defined by a personal history of respiratory allergy, together with positive SPT to several common aeroallergens. Of the atopic group, 17 patients (48.6%) suffered from rhinitis and bronchial asthma, 12 (34.3%) showed only rhinitis, two had only bronchial asthma (5.7%), and in four cases (11.4%) both atopic dermatitis and respiratory symptoms were present. In 29 of them (82.9%), respiratory symptoms preceded food allergy onset, suggesting a primary sensitization via inhalant route. Total IgE ranged from 12 to >2000 kU/l, with a geometric mean of 250.4 kU/l, and it was within normal limits (<100 kU/l) in nine patients (23.7%).

Clinical manifestations of mustard allergy reported by patients consisted of OAS in 18 cases (47.4%), urticaria/angioedema in 16 (42.1%), and systemic anaphylaxis in

four (10.5%) – one of them with mustard-dependent exercise-induced anaphylaxis. Mustard SPT was positive in all patients, with a wheal (mean $\pm$ SD) of 6.7 ± 3.6 mm, being negative in all control subjects. Specific IgE to mustard showed a positive result in 35 patients (92.1%), with a geometric mean of 1.7 kU/l, ranging from <0.35 to 24.0 kU/l, and being negative in controls (Table 1).

Double-blind food challenges

Fourteen patients did not undergo DBPCFC because of either severe symptoms ($n = 4$) or because of denial of consent. The remaining 24 patients underwent DBPCFC, with all of them positive for prick-prick test to the mustard sauce used for oral challenge, with a mean wheal diameter ($\pm$ SD) of 6.5 ± 2.6 mm (range 3–14 mm), and no significant differences with commercial mustard SPT results. In 14 cases (58.3%), mustard challenge was positive, with OAS being the most frequent symptom observed [in 10 patients (71.4%)]. They showed pruritus and mild angioedema of the lips, tongue, palate and throat, followed by a rapid resolution of symptoms. Nevertheless, one patient showed angioedema and bronchial asthma after mustard ingestion, meanwhile patient no. 15 reacted with systemic anaphylaxis, with a significant rise in tryptase levels from 11.4 μ g/l at baseline to 23.6 μ g/l. Although showing highly positive mustard SPT and specific IgE results, this patient referred only OAS in his clinical history. All patients completely improved after symptomatic treatment in <90 min.

Mean cumulative reactive dose of mustard sauce ($\pm$ SD) was 891.4 ± 855.2 mg, equivalent to 124.8 ± 119.7 mg of mustard, and no significant relation with mustard SPT or specific IgE could be demonstrated. When comparing commercial mustard extract SPT results between truly allergic patients (positive DBPCFC) and sensitized patients (negative DBPCFC), a statistically significant difference ($P < 0.05$) was found (8.2 ± 3.7 vs 5.3 ± 2.4 mm). The area under the ROC curve was 0.736, with a 95% confidence interval (CI) of 0.518–0.892. The best cut-off value for mustard commercial SPT was 8 mm, with a specificity of 90% (95% CI, 55.5–98.3), and a sensitivity of 50% (95% CI, 23.1–76.9) for predicting a positive challenge outcome. No significant differences were found between both subgroups of patients, regarding either prick-prick or specific IgE tests to mustard. Four of the 10 patients with negative DBPCFC wished to eat mustard, and they were subsequently open-challenged with up to 25 g of mustard sauce in a hamburger, with good tolerance in all cases.

Aeroallergen sensitizations

Diverse mustard-associated aeroallergen sensitizations were found in mustard-hypersensitive patients (Table 2).

Table 1. Clinical characteristics of 38 patients with mustard IgE-mediated hypersensitivity

No.	Age/sex	Atopic symptoms	Total IgE (kU/l)	Symptoms reported	Mustard		
					SPT/prick-prick*	Specific IgE (kU/l)	DBPCFC (symptoms–dose†)
1	24/F	r, BA	1234	U/AE	3/4	1.17	Negative
2	18/F	r, BA	12	OAS	14/6	1.68	(OAS-44.8)
3	23/F	r	361	OAS	5/6	0.85	(OAS-44.8)
4	39/F	r	294	OAS	3/5	0.95	Negative
5	14/F	r	350	U/AE	5/4	7.69	Negative
6	26/F	r	57	OAS	5/6	1.74	(OAS-44.8)
7	25/M	—	49	OAS	4/6	1.22	Negative
8	31/M	r, BA	158	U/AE	10/9	0.75	(OAS-156.8)
9	29/F	r, BA, ad	221	OAS	3/6	<0.35	(OAS-44.8)
10	15/F	r, ad	95	U/AE	8/6	0.46	Negative
11	24/M	r, BA	115	U/AE	3/6	<0.35	Negative
12	15/M	r, BA, ad	1516	OAS	10/9	1.73	(OAS-492.8)
13	28/F	r, BA	322	U/AE	7/5	1.74	Negative
14	17/M	r	78	U/AE	10/5	0.45	Negative
15	27/F	r, BA	241	OAS	15/13	24.00	(A-156.8)
16	15/M	r, BA	212	U/AE	7/4	0.91	(U-44.8)
17	11/M	r	82	OAS	3/3	1.10	(OAS-156.8)
18	26/F	r	596	U/AE	7/nd	0.50	nd
19	8/M	r	309	U/AE	6/nd	3.34	nd
20	9/M	r, BA	>2000	A	8/nd	9.75	nd
21	27/F	r, BA	681	OAS	4/nd	2.06	nd
22	6/M	r, BA, ad	>2000	U/AE	3/nd	4.69	nd
23	15/M	BA	130	OAS	12/nd	2.35	nd
24	3/M	r, BA	308	U/AE	4/nd	0.98	nd
25	29/M	r	314	U/AE	3/nd	6.22	nd
26	16/M	r	739	OAS	9/5	7.49	(OAS/C-156.8)
27	24/F	BA	895	OAS	6/6	1.30	Negative
28	23/F	r	256	OAS	4/8	2.01	Negative
29	20/F	—	45	OAS	16/nd	0.70	nd
30	33/F	r, BA	113	A	4/nd	2.06	nd
31	35/F	r, BA	36	OAS	10/6	2.42	(OAS-156.8)
32	28/M	r	294	OAS	7/14	17.00	(OAS-44.8)
33	37/F	r, BA	>2000	U/AE	11/8	1.99	(AE/AS-156.8)
34	29/M	r, BA	448	OAS	6/5	1.85	(OAS-44.8)
35	28/F	—	39	EIA	5/nd	<0.35	nd
36	16/M	r, BA	329	U/AE	5/nd	0.52	nd
37	23/F	r, BA	650	A	3/nd	7.71	nd
38	16/M	r, BA	329	U/AE	5/nd	0.52	nd

No., patient number; F, female; M, male; r, rhinitis; BA, bronchial asthma; ad, atopic dermatitis; U, urticaria; AE, angioedema; OAS, oral allergy syndrome; A, anaphylaxis; EIA, exercise induced anaphylaxis; nd, not done; DBPCFC, double-blind placebo-controlled food challenge; C, conjunctivitis.

* Expressed in millimetre of mean wheal diameter 15 min after puncture, performed with a mustard commercial extract (SPT) or with a commercial mustard sauce (prick-prick).

† Dose–response expressed in milligrams of mustard.

Surprisingly, 37 of the 38 patients (97.4%) were sensitized to *A. vulgaris* pollen, which is rather a rare cause of respiratory allergy in our area, and 34 of them also showed a positive mugwort-specific IgE determination, with a geometric mean value of 2.9 kU/l (ranging from 0.35 to 83.5). This association was highly significant, when compared with the paired control group ($P < 0.001$). Thirty-five patients (92.1%) were also sensitized to other pollens besides mugwort although at lower rates, including weeds such as *Chenopodium* and *Chrysanthemum* (26 cases each); grasses in 20 subjects, such as *Poa* ($n = 14$), *Lolium* ($n = 13$) and *Anthoxanthum*

($n = 13$); and trees in 28 cases, such as *Ulmus* ($n = 25$) and *Platanus* ($n = 15$).

With respect to other aeroallergens, 26 patients (68.4%) were sensitized to mites, both to *Dermatophagoides* and to storage mites, and 22 (57.9%) to animal – cat and dog epithelia – which are in fact the most frequent aetiological agents of respiratory allergy in our area. Six subjects (15.8%) showed fungus sensitization, five of them to *A. alternata*. Another four patients (10.5%) showed latex sensitization, which was symptomatic in two cases. There were no significant differences in sensitizations to fungi, animals or latex between study and control

Table 2. Aeroallergen and plant-derived food sensitizations in a group of 38 patients with mustard IgE-mediated hypersensitivity

No.	Mugwort SPT (mm)	Mugwort IgE (kU/l)	Other pollens	Other aeroallergens	Plant-derived foods
1	10	64.70	w, g, t	Mites, animals	b, n, l, r
2	4	0.76	w, t	—	b, n, l, r, c
3	7	3.29	w, t	—	b, n, l, r
4	3	<0.35	w, t	Mites, fungi, animals	b, n, l, r
5	5	42.90	w, t	Mites, animals	b, n, l, r
6	6	5.26	w, g, t	—	b, n, l, r
7	3	1.44	w, t	—	b, n, l, r
8	1	<0.35	—	Mites, animals	b, n, l, r, c
9	4	1.15	w, t	—	b, n, l, r
10	6	7.75	w, t	Mites	b, n, l, r, c
11	4	<0.35	w, t	Mites, animals	b, n
12	6	3.79	w, g, t	Mites, animals	b, n, l, r
13	5	0.64	w, t	Mites, animals, latex	b, n, l, r
14	5	4.01	w	Fungi	b, n, l, r, c
15	10	3.51	w, g	Animals, latex	b, n, l, r
16	6	0.77	w, t	—	b, n, l, r
17	3	<0.35	—	Mites	b, n, l, c
18	20	4.63	w, g, t	Mites, animals	b, n, l, r
19	8	21.10	w, g	Mites, fungi, animals	b, n, l, r
20	5	24.60	w, g, t	Mites, fungi, animals, latex	b, n, l, r
21	7	23.70	w, t	Mites, animals	b, n, l, r
22	9	83.50	w, t	Mites, animals	b, n, l, c
23	3	0.55	w, g, t	Fungi, animals	b, n, l, r
24	4	2.67	w, g, t	Mites, animals	b, n, l, r
25	7	1.40	w, g, t	Mites	b, n, l, r
26	9	0.46	w, g, t	Mites, animals	b, n, l, r, c
27	5	15.00	w, g, t	Mites, fungi, animals	b, n, l, r
28	3	0.65	w, g	Mites	b, n, l, r
29	8	2.62	w, g, t	—	b, n, l, r
30	4	2.16	w, t	Mites, animals	b, n, l, r
31	4	82.80	w, g, t	Mites	b, n, l, r
32	4	0.69	w, g, t	—	b, n, l, r
33	4	0.57	w, g	Mites, animals, latex	b, n, l, r
34	5	1.00	w	Mites	b, n, l, r
35	3	3.16	—	—	b, n, l, r
36	3	2.08	w, g, t	Mites, animals	b, n, l, r
37	5	28.10	w, g, t	Mites, animals	b, c
38	3	2.08	w, g, t	Mites, animals	b, n, l, r

No., patient number; w, weeds excluding mugwort; g, grasses; t, trees; b, Brassicaceae family excluding mustard; n, nuts excluding almond and peanut; l, leguminous including peanut; r, Rosaceae family including almond; c, corn.

groups. No pollen sensitization was found among control subjects.

Food sensitizations

Mustard-hypersensitive patients also showed a wide range of food-associated sensitizations, reaching as much as 578 in the whole patient group, with clinical manifestations in up to 242 cases (41.9%). These manifestations comprised of mild symptoms such as OAS (48.8%) to more severe symptoms like urticaria/angioedema (40.1%), anaphylaxis (3.7%), food-dependent exercise-induced anaphylaxis in six patients (2.5%), or bronchial

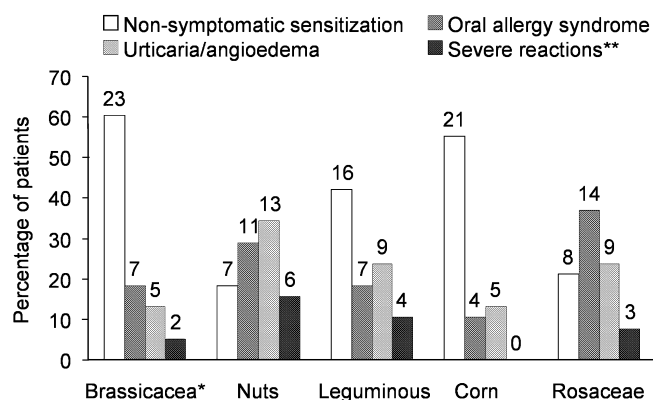


Figure 1. Food-associated sensitizations in a group of 38 patients with mustard IgE-mediated hypersensitivity. Results are expressed in percentage of patients (relative frequency). Number of patients in each group is shown at the top of the columns. For calculation purposes, neither peanut nor almond were included in the nuts group, because they were in leguminous family and Rosaceae fruits, respectively. *Excluding mustard, **including bronchial asthma, systemic anaphylaxis and exercise-induced anaphylaxis.

asthma (5%). Plant-derived foods most commonly involved are summarized in Table 2, and the clinical symptoms described in Fig. 1.

All our patients (100%) showed associated sensitization to vegetables belonging to Brassicaceae family excluding mustard, 15 of them with clinical symptoms. Nine of these 15 patients were allergic to both cabbage and cauliflower, five of them only to cabbage, and only one patient showed clinical manifestations not only to cabbage and cauliflower, but also to broccoli. One case of systemic anaphylaxis and another of exercise-induced anaphylaxis were reported, both involving uncooked cabbage consumption. These sensitizations were further confirmed by determining specific IgE to cabbage, cauliflower and broccoli, which was positive in 37 of the 38 patients, with geometric mean values (ranges) of 4.3 (0.35–76.6), 5.8 (0.35–89.8) and 4.6 kU/l (0.35–69.3), respectively. Specific IgE to cabbage, cauliflower and broccoli results were negative in control subjects.

Other food-associated sensitizations comprised of nuts – excluding peanut and almond – in 37 patients (97.4%), 30 of them with clinical allergy; to leguminous – including peanut in this group – in 36 patients (94.7%), 20 of them symptomatic; corn – with no other cereals – in 30 patients (78.9%), nine of them allergic; Rosaceae fruits – including almond – in 34 patients (89.5%), 26 of them reporting symptoms on ingestion; and to several other foods at lower sensitization rates. All these food-associated sensitizations detailed were highly significant ($P < 0.001$) when compared with the control group.

Exercise-induced anaphylaxis was diagnosed in six patients, but food challenges with or without exercise

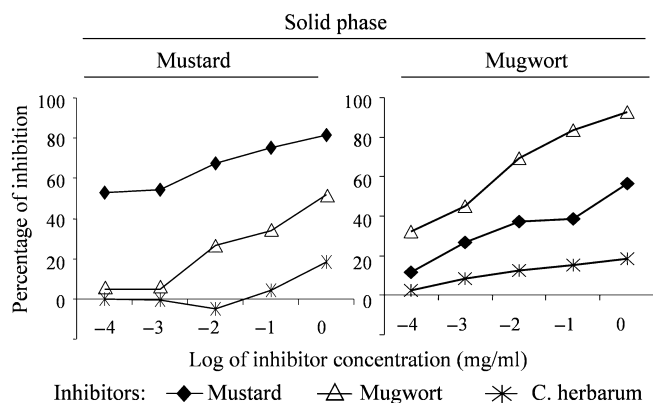


Figure 2. Reciprocal CAP inhibition assays between mustard and mugwort pollen, using a pool of sera from five mustard allergic patients, and a *C. herbarum* commercial extract as negative control. Inhibitions higher than 50% were reached at maximum concentrations of either mustard or mugwort, when used as inhibitors of the respective counterpart in solid phase, meanwhile inhibitions with control extract were lower than 20%.

were not performed because of the severity of reactions. Foods involved were peach, peanut, walnut, banana, sunflower seed, and one patient reacted to both mustard and cabbage in different events. The time lapse between food consumption and exercise-induced reaction ranged from 30 to 90 min, and the type of exercise involved was a game of football in three patients, jogging in two, and gymnasium exercise in another.

CAP inhibition assays

Results of CAP inhibition assays with a pool of sera from five mustard allergic patients are shown in Fig. 2. When used as inhibitors, both mustard and mugwort extracts manage to inhibit >50% – at maximum concentrations – the IgE binding to their correspondent counterpart in solid phase. We have further investigated reciprocal mustard–mugwort maximum inhibitions with each of those five sera. When mustard was used in solid phase, mugwort pollen maximum inhibition levels ranged from 28 to 100%, meanwhile with mugwort pollen in solid phase, mustard maximum inhibition rates varied from 19 to 83%, thus demonstrating a high individual cross-reaction variability, which could explain the partial reciprocal cross-inhibition when using this pool of sera.

In the same way, results of CAP inhibition experiments with individual sera from 11 patients allergic to cabbage, cauliflower and broccoli are shown in Fig. 3. Mustard commercial extract manage to inhibit from 28 to 88% the IgE-binding to the respective Brassicaceae in solid phase, meanwhile mugwort pollen did from 32 to 90%.

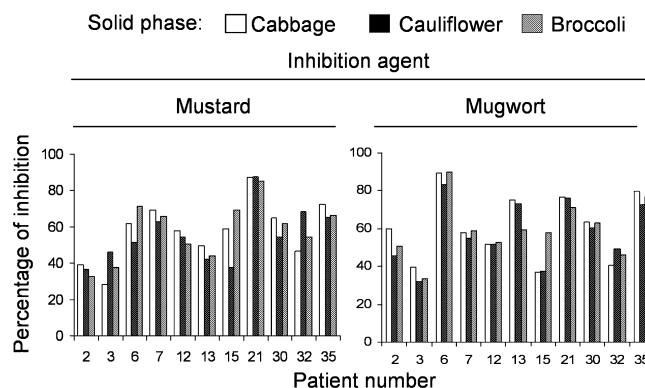


Figure 3. CAP inhibition assays performed with individual sera from a subgroup of 11 patients who were allergic not only to mustard, but also to cabbage, cauliflower and broccoli. When using each one of these Brassicaceae in solid phase, mustard commercial extract manage to inhibit from 28 to 88%, meanwhile mugwort pollen commercial extract did from 32 to 90%. A *C. herbarum* commercial extract, used as negative control, showed inhibitions lower than 10%.

Discussion

Although IgE-mediated hypersensitivity to mustard is considered to be a very uncommon problem, a group of 38 patients is here described, and we have demonstrated a significant association of this condition with sensitizations to mugwort pollen, to other members of the Brassicaceae family, and to several unrelated plant-derived foods. In contrast with previous reports (7), mustard allergy is more frequent among adult patients in our area. Moreover, meanwhile atopic dermatitis has been described as the most common clinical manifestations of mustard allergy in children, and with no anaphylaxis observed at this age group (8), we have found that OAS is very frequent among adults, with systemic anaphylaxis in as much as 10% of them. This fact suggests that, as previously reported with other foods (18), children may sensitize to mustard via the oral route, meanwhile adult patients could sensitize to a cross-reacting aeroallergen by the respiratory route. Prospective epidemiological studies are needed to fully confirm these data.

Nowadays, DBPCFC is the gold standard for food allergy diagnosis (8, 10). However, in the particular case of mustard, it is not easy to appropriately mask its strong taste. We have managed to do it by using a yoghurt-based vehicle with vanilla–lemon flavouring, reaching a total final dose of 10 g of mustard sauce. Meanwhile Rance et al. (8) have found a 42% rate of mustard allergy in children sensitized to mustard, by using SBPCFC, Morisset et al. (10) have observed a rather lower rate (23.3%), by performing single- and double-blind placebo-controlled food challenges. In our study, almost 60% of the mustard DBPCFC showed positive results, a rate considerable higher than previously reported, which could be related to both patient inclusion criteria and DBPCFC method.

Discrepancies between clinical history and DBPCFC results, regarding both outcome and severity of food-induced adverse reaction, agree with previous data reported on DBPCFC performed with other plant-derived foods (19, 20). It is remarkable that we have demonstrated a significant relationship between SPT mean wheal diameter, performed with a mustard commercial extract, and challenge outcome, obtaining a threshold value of 8 mm, with a specificity of 90% and a sensitivity of 50%.

It is well known that patients with respiratory allergy to pollens frequently show significantly associated IgE-mediated hypersensitivities to certain foods, mainly fruits and vegetables (21, 22). These associated allergies can be very important from the clinical point of view, and are sometimes referred to as 'syndromes'. An example is the so-called celery–mugwort–birch–spice syndrome, described in Center Europe, and defined by the association between mugwort pollinosis, birch pollinosis and certain food allergies – mainly to celery and spices (23–26). The immunopathological basis for these clinical associations is the cross-reactivity among antigens from different sources, because of molecular similarities among their epitopes (21, 22). The ubiquitous profilin family, as well as food homologues of Art v 1 – a major mugwort allergen in the MW range of 60 kDa – are potentially responsible for this celery–mugwort–birch–spice syndrome (24).

Caballero et al. (9, 22) mentioned a possible association between mugwort pollinosis and mustard – among several other foods – allergy. However, Rance et al. (8) did not find such associations with pollens or foods in a group of 36 children sensitized to mustard. A recent study on mustard allergy confirmed by food challenges has not focused on possible associations to pollen or other food allergies (10). Our series of patients showed not only a highly significant association between mustard and mugwort allergies, but also among mustard allergy and several other unrelated plant-derived food immediate hypersensitivities. In fact, nuts, legumes, Rosaceae fruits, and corn allergies are also involved. Moreover, partial cross-reactivity between mustard and mugwort pollen was confirmed by CAP-inhibition assays. Therefore, although a primary sensitization to other cross-reacting pollen has not been ruled out, our data suggests the existence of a so far not described mugwort–mustard allergy syndrome, which probably involves various taxonomically unrelated plant-derived food families. Both profilins and homologues of Art v 1 could be responsible for this syndrome, as well as lipid transfer proteins, which have been recently described as allergens of Rosaceae fruits and mugwort pollen (27), and studies – including immunoblot analysis – should be performed to further characterize the antigens involved.

Possible cross-reactivity of mustard with other members of the Brassicaceae family has been suggested, but some authors could not confirm this hypothesis (4, 5), and therefore it is considered to be a rare condition (8, 28). However, a patient with anaphylaxis to cabbage and positive skin tests not only to this food, but also to mustard,

cauliflower and broccoli has been described (29). What we have found is that all our mustard allergic patients were sensitized to other members of the Brassicaceae family, and approximately 40% of them were truly allergic to at least one of them, suggesting that cross-reactions between mustard and taxonomically related foods are very common and clinically relevant. Furthermore, we have confirmed cross-reactivity among mustard, mugwort pollen and other Brassicaceae vegetables by CAP-inhibition assays.

Food-dependent exercise-induced anaphylaxis is a well-defined disorder that is increasingly being recognized, which is usually triggered by specific foods (30). So far, wheat allergy has been the most frequently implicated, although at times no specific food is identified. To our knowledge, neither mustard nor mugwort allergies have been previously related to food-dependent exercise-induced anaphylaxis. In this context, it is noteworthy that six of our mugwort–food allergic patients suffered seven episodes of specific food-dependent exercise-induced anaphylaxis, in one case directly induced by mustard ingestion, although we did not confirm this diagnosis by specific challenge due to severity of reactions. This suggests that the allergens responsible for the mugwort–mustard allergy syndrome are at the same time implicated in most cases of food-dependent exercise-induced anaphylaxis, at least in our area.

In our opinion, the mugwort–mustard allergy syndrome here proposed could have clear clinical implications. Thus, every patient allergic to mugwort should be asked about adverse reactions to mustard and other foods, and skin tested with a battery of plant-derived foods. At the same time, every patient allergic to mustard must avoid other members of the Brassicaceae family, until tolerance is demonstrated by the corresponding study. In these patients, respiratory symptoms during pollen season and adverse reactions to other plant-derived foods should be ruled out. Finally, mugwort–mustard and related food allergies must be considered in patients with food-dependent exercise-induced anaphylaxis.

In conclusion, mustard IgE-mediated allergy is a not uncommon disorder that can induce severe adverse reactions, and frequently involves other members of the Brassicaceae family. A significant association with both mugwort pollinosis and several unrelated plant-derived food allergies is demonstrated, constituting a novel mugwort–mustard allergy syndrome. At the same time, an intriguing association of this syndrome with food-dependent exercise-induced anaphylaxis is here described.

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