

## Original article

# Prospective study of mustard allergy: first study with double-blind placebo-controlled food challenge trials (24 cases)

**Background:** Mustard allergy accounts for 1.1% of food allergies in children. However, double-blind placebo-controlled food challenge trials (DB PCFCs) have not yet been proposed.

**Objective:** To carry out DB PCFCs to determine the real frequency of mustard allergy in patients sensitized to mustard.

**Methods:** A prospective study was conducted in 30 subjects aged 3–20 years presenting positive prick tests to ground mustard seeds (*Brassica nigra*), mustard flour (*B. juncea*), metabisulfite-free strong mustard seasoning (*B. juncea*) and a commercialized allergenic extract (*B. nigra*). Twenty-seven subjects were screened for mustard-specific immunoglobulin E (IgE). PCFCs were carried out either DB or single blind (SB) with up to 1340 mg of metabisulfite-free seasoning.

**Results:** The mean diameter of the wheal induced by prick tests with the allergenic extract was lower (n.s.) than that induced by the native mustard products: 5.8 mm (1.5–15) vs 6.9 mm (0.5–18) for *B. nigra* ground seeds, 7.8 mm (1–20) for *B. juncea* flour and 9.7 mm (3–20) for the strong mustard seasoning. The diameter of the wheal induced by the allergenic extract was significantly different from that induced by the mustard seasoning ( $P < 0.005$ ). The mean of mustard specific-IgE values was 8.7 KU/l (0.35–72.4). Seven of 30 food challenges were considered positive. Mean prick test results in the positive and negative PCFC subgroups were 5.5 mm vs 5.9 mm for the commercialized extract, 10.9 mm vs 5.8 mm for *B. nigra* ground seeds ( $P < 0.01$ ), 9.9 mm vs 7.1 mm for *B. juncea* flour (n.s.  $P > 0.25$ ) and 11.5 mm vs 9.1 mm for the metabisulfite-free mustard seasoning (n.s.  $P > 0.1$ ). Mean specific IgE values determined by CAP system radioallergosorbent test (Phadebas Pharmacia) were higher but not significantly so ( $P > 0.25$ ) in the subgroup with mustard allergy (12.3 K/l vs 7.6 KU/l).

**Conclusions:** About 23.3% of the sensitized subjects were allergic to a routine dose of mustard. Positive prick tests and the presence of specific IgE were not predictive. SB PCFC or DB PCFC is required before recommending avoidance diets.

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Mustard-dependent immunoglobulin E (IgE) anaphylaxis which may be severe, has been reported over the last 20 years (1–7). Mustard is present in an increasing number of industrial preparations and can be a masked allergen, giving rise to serious safety issues (1, 2, 5, 8).

Mustard allergy is the most frequent spice allergy. Where spice allergy is suspected, skin prick tests (SPT) and spice- and seasoning-specific IgE screening generally reveal sensitization to mustard (9). Of the 707 children under 15 years with 689 food allergies, allergy to mustard was recorded in 1.1% (10), representing one of four sensitized to mustard. The rate was 0.84% in 196 adults. Other series report from 3 to 8.9% of cases (11–13).

The need to reinforce food safety considerations led the European Community to suggest a modification to directive no. 2001/13/CE, on September 6 2001 regarding

the mandatory labeling of 10 allergens considered the most common or the most hazardous. These proposals took into account recommendations drawn up from a literature review of cases of food allergy with double-blind, placebo-controlled food challenges (DB PCFCs) (14). Mustard allergy was not diagnosed, in the absence of such DB PCFCs.

The primary objective of this 2-year prospective study was to develop a procedure for blind food challenges. The secondary objective was to define the relationship between sensitization and mustard allergy in patients undergoing investigation for food allergies. Thirty subjects (28 children and two adults) were selected on the basis of positive prick tests. A single-blind, SB PCFC or a DB PCFC showed that 23.3% of sensitized individuals were allergic to mustard.

## Material and methods

### Subjects

Thirty subjects were included: 28 children and two adults. Food allergies were suspected on the basis of urticaria and angioedema, atopic dermatitis, asthma, abdominal pain, and bouts of diarrhea. Clinical reactions to mustard clearly identified at the interview were not taken into account for inclusion, which was based solely on prick tests.

### Skin prick tests

The positive control was 9% codeine sulfate. Prick-in-prick tests were performed with 25 natural foods (15). The positive criterion was a wheal diameter of more than or equal to the 9% codeine phosphate control. The diameter of a SPT (with a 0.5 mm precision) is an average between the largest and smallest wheal diameters.

The materials tested were *Brassica nigra* ground seeds (pharmaceutical industry), *B. juncea* flour (food industry) according to Niinimäki (16), in, respectively, 27 and 29 subjects. Prick tests with a seasoning (metabisulfite-free strong mustard supplied by the food industry) from *B. juncea* (33.6% of the compound) were also carried out in 27 subjects. Sixteen subjects underwent prick tests with a *B. nigra* extract (Allerbio Laboratory, Varennes en Argonne, France).

### Specific IgEs

Screening for mustard-specific IgEs was carried out on 27 sera using Cap system RAST (Phadebas; Pharmacia, Uppsala, Sweden) (*Brassica* and *Sinapis* spp).

### Food challenges

Twenty-four subjects participated in DB PCFCs to mustard. SB PCFC were carried out in six other cases.

The seasoning (*B. juncea* seed) was supplied by the food industry. The seasoning contained 33.6% of mustard seeds and was free of metabisulfite or other spice or flavoring. It was masked in a very cold drink such as Coca-Cola® or Pepsi-Cola®. The vehicle was used as a placebo and taken through a straw, thus reducing the likelihood of differentiating the placebo from the test drink. The preparation was made just before drinking so that the characteristic taste (allyl isothiocyanate) did not have time to develop. A preliminary study in volunteers demonstrated that in these conditions the amount of mustard (1340 mg) could not be identified. This amount was selected on the basis of routine consumption.

Increasing doses (10, 30, 100, 300, and 900 mg) were administered every 20 min ensuring a cumulative dose of 1340 mg. There was a 24-h interval between administration of the mustard and the placebo. In six cases, a SB PCFC was performed first with mustard and then with placebo, in order to reduce follow-up.

### Statistical analysis

Mean diameters of the wheal and specific IgE levels were compared using a Student's test ( $n \leq 30$ ).

## Results

Eleven females and 19 males were included: 28 children from 3 to 15.5 years and two adults of 20 years old.

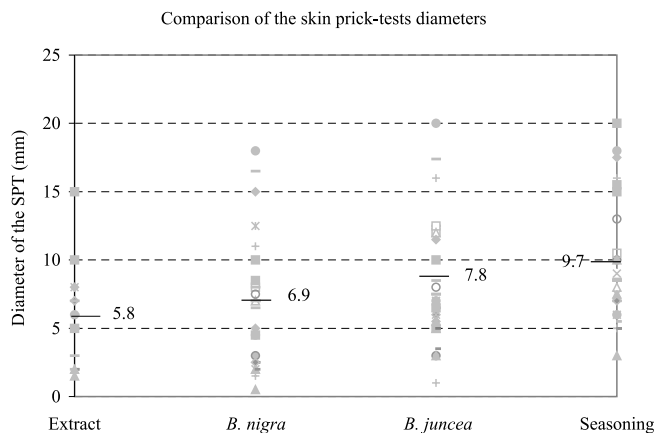


Figure 1. Skin prick tests diameters with ground *Brassica nigra* seeds, *B. juncea* flour, seasoning (*B. juncea*) and *B. nigra* extract.

Prick tests with different mustards (Fig. 1) showed increasing reactivity in the following order: 5.8 mm (1.5–15) for the commercialized extract, 6.9 mm (0.5–18) for *B. nigra* ground seeds, 7.9 mm (1–20) for *B. juncea* flour, and 9.7 mm (3–20) for the metabisulfite-free strong mustard seasoning. The difference in mean diameters of wheals induced by prick tests with the commercialized extract on the one hand, and with the strong mustard seasoning on the other hand, were statistically significant ( $P < 0.005$ ).

The mean value of mustard-specific IgE was 8.7 IUK/l (0.35–72.4), while the mean value of rapeseed pollen-specific IgE was 0.6 IUK/l (0.35–1.80).

Seven SB PCFC or DB PCFC were positive (eczema, conjunctivitis, abdominal pain and diarrhea, palpebral pruritus, sneezing and erythema, wheezing) (Table 1). Challenge was discontinued after 1 h in patient 3 because of the onset of rash on eczema. Patient 4 presented rhinitis and urticaria 20 min after the second dose. Patient 10 reacted at the last dose, 2 h after starting the challenge. Conjunctivitis and abdominal pain followed by diarrhea 1 h later were observed. Patient 14 presented with onset of facial pruritus, rash and rhinorrhea 20 min after the last dose of mustard (900 mg). Patient 30 presented minor flexural eczema exacerbation 8 h after the challenge. Patient 25 experienced wheezing during the challenge. The signs observed in subject 27 were slight (velum palati pruritus and sneezing, at the limit of the routine irritative signs documented for anybody eating mustard).

In nonallergic subjects mean results to prick tests were 6.0 mm for the allergenic extract, 5.8 mm for *B. nigra* ground seed, 7.1 mm for *B. juncea* flour and 8.7 mm for the metabisulfite-free strong mustard seasoning.

In the subgroup of seven allergic subjects, the mean results to prick tests were 5.5 mm for the allergenic extract, 10.9 mm for *B. nigra* ground seeds, 9.9 mm for *B. juncea* flour and 11.5 mm for the metabisulfite-free

Table 1. Clinical and biological results in 30 subjects sensitized to mustard.

| No | Age | Clinical history | Prick-in-prick (mm) |                  |           | SPT extract (mm)<br><i>B. nigra</i> | Specific IgE (KUI/l) |                 | SB or DB PCFC (1340 mg) | Symptoms                                       |
|----|-----|------------------|---------------------|------------------|-----------|-------------------------------------|----------------------|-----------------|-------------------------|------------------------------------------------|
|    |     |                  | <i>B. nigra</i>     | <i>B. juncea</i> | Seasoning |                                     | Mustard              | Rapeseed pollen |                         |                                                |
| 1  | 3   | AD               | 2                   | 5                | 7.5       | 1.5                                 | 2.33                 | n.d.            | DB negative             |                                                |
| 2  | 3   | AD               | 5                   | n.d.             | n.d.      | n.d.                                | n.d.                 | n.d.            | DB negative             |                                                |
| 3  | 3   | AD, A            | 8.5                 | 5                | 15.5      | 10                                  | <0.35                | n.d.            | DB positive (440 mg)    | Rash on flexural eczema<br>Rhinitis, urticaria |
| 4  | 3   | DA, U            | 15                  | 11.5             | 17.5      | 8                                   | 9.28                 | <0.35           | DB positive (40 mg)     |                                                |
| 5  | 3   | AD, AO           | 7                   | 7                | 9         | n.d.                                | 72.4                 | 0.91            | DB negative             |                                                |
| 6  | 3   | AD, Dg S         | 7                   | 7.5              | 9         | 3                                   | 1.96                 | <0.35           | SB negative             |                                                |
| 7  | 3   | AD               | 3                   | 3                | 10        | n.d.                                | <0.35                | n.d.            | DB negative             |                                                |
| 8  | 3   | AD               | 8                   | 12               | 10.5      | n.d.                                | 7.69                 | n.d.            | DB negative             |                                                |
| 9  | 4   | AD, A, U         | 5                   | 5                | 5         | 5                                   | <0.35                | n.d.            | DB negative             |                                                |
| 10 | 4   | AD, A            | 16.5                | 12.5             | 8.5       | 2                                   | 43.4                 | 1.80            | SB positive             | Conjunctivitis, abdominal pain, diarrhea       |
| 11 | 4   | AD, A            | 2.5                 | 6                | n.d.      | 7                                   | 4.8                  | n.d.            | DB negative             |                                                |
| 12 | 4   | A                | n.d.                | 12.5             | n.d.      | n.d.                                | 6.48                 | <0.35           | DB negative             |                                                |
| 13 | 5   | DA, A            | 2                   | 3.5              | 5         | 2                                   | 1.23                 | n.d.            | DB negative             |                                                |
| 14 | 5   | AD, Abd P        | 7                   | 12               | 8         | n.d.                                | n.d.                 | n.d.            | DB positive             | Pruritus, rash, rhinorrhea                     |
| 15 | 6   | AD               | 6.5                 | 7.5              | 7         | 7                                   | 24.1                 | 0.77            | DB negative             |                                                |
| 16 | 6   | AD               | 12.5                | 7                | 7.5       | 8                                   | 0.47                 | <0.35           | DB negative             |                                                |
| 17 | 6   | AD               | 4.5                 | 7                | 6         | 6                                   | 5.98                 | <0.35           | DB negative             |                                                |
| 18 | 7   | AD               | 1.5                 | 1                | 5         | n.d.                                | 4.06                 | 1.41            | SB negative             |                                                |
| 19 | 7   | AD               | 7                   | 6.5              | 5.5       | 6                                   | 1.24                 | 1.23            | DB negative             |                                                |
| 20 | 7   | A, U, AO Dg S    | 8                   | 8.5              | 8.5       | n.d.                                | 5.6                  | <0.35           | DB negative             |                                                |
| 21 | 8   | U                | 7                   | 5                | 8.5       | 5                                   | <0.35                | <0.35           | DB negative             |                                                |
| 22 | 8   | DA, A, AO        | 4.5                 | 6.5              | 15        | 5                                   | 1.76                 | n.d.            | DB negative             |                                                |
| 23 | 8   | DA, A            | 7.5                 | 8                | 13        | n.d.                                | <0.35                | n.d.            | DB negative             |                                                |
| 24 | 9   | AO, A            | 10                  | 10               | 20        | 15                                  | 10.4                 | 1.31            | DB negative             |                                                |
| 25 | 10  | AD, A            | n.d.                | 3                | 10        | n.d.                                | 2.46                 | 0.73            | SB positive             | Sibilant rales                                 |
| 26 | 11  |                  | 2                   | 6                | 6         | n.d.                                | <0.35                | <0.35           | DB negative             |                                                |
| 27 | 11  | AO, A            | 18                  | 20               | 18        | n.d.                                | 17.4                 | <0.35           | SB positive             | Pruritus, sneezing                             |
| 28 | 15  | AO, A            | n.d.                | 5.5              | 7         | n.d.                                | 0.46                 | <0.35           | SB negative             |                                                |
| 29 | 20  | AD, A            | 11                  | 16               | 16        | n.d.                                | n.d.                 | n.d.            | DB negative             |                                                |
| 30 | 20  | DA, A            | 0.5                 | 5.5              | 3         | 2                                   | 0.79                 | <0.35           | DB positive             | Minor eczema exacerbation                      |

A: asthma; Abd P: abdominal pain; AD: atopic dermatitis; AO: angioedema; Dg S: digestive symptoms; SPT: skin prick test; U: urticaria; n.d.: not done.

seasoning. The difference in mean results of prick tests to *B. nigra* seeds was statistically significant between the two groups ( $P < 0.005$ ). It was not significant for the allergenic extract, for *B. juncea* ( $P > 0.05$ ) and for *B. juncea* seasoning ( $P > 0.1$ ).

Mean mustard CAP-RAST RIA values were 12.3 IUK/l in the subgroup allergic to mustard vs 7.6 IUK/l in the nonallergic group. These differences were not statistically significant ( $P > 0.25$ ). Mustard RAST inhibition was carried out on five sera, showing a nonsignificant inhibition by rape pollen (Table 1).

## Discussion

Mustard is a cruciferous plant, a genus of the Brassicaceae family, which also includes rape, cabbage, turnips, etc. Strong mustard is extracted from *B. juncea*, a plant rich in allyl isothiocyanate. The *Sinapis alba* species, rich in parahydroxybenzoyl isothiocyanates, is used for some mustard seasonings and English mustard. *Brassica nigra* has a more acid, bitter taste and is used

in the pharmaceutical industry for its revulsive properties (17).

The major allergen of mustard is a 2S albumin, a storage protein still referred to as napin, composed of two chains, one heavy and one light (39 and 88 amino acids) linked by two disulfide bridges. Rape oil, leguminous plants (peas and soya) and sesame allergens, as well as the major allergen in Brazil nuts, all belong to this protein family (18, 19).

*Sin a1* was cloned recently (20). The principal allergen of *B. juncea* is called *Bra j1* and is very close to *Sin a1* (21). There is marked *in vitro* cross-reactivity between the principal allergen of rapeseed *Bn III* and the mustard allergen *Sin a1* (22). Study of *B. campestris* pollen allergens, from which rape issues, evidences a major 14 kD allergen. Allergenic proteins with the same molecular mass were identified in *S. alba* (23). A cross-sensitivity with rapeseed has been shown in the case of anaphylaxis induced by mustard (24).

Sensitization has been detected by various prick tests using allergenic extracts (3), a marketed mustard (6) and ground seeds (24).

Comparison of tests with an allergenic extract of *B. nigra* and with *B. nigra* seeds showed no significant difference. Mustard from *B. juncea* was significantly more reactive than these former products, according to previous observations (16).

There is no difference between *B. juncea* flour and *B. juncea* seasoning, suggesting that soaking seeds in vinegar does not enhance the allergenicity. Prick tests with *B. juncea* flour and seasoning were not different. Previous testing of volunteers showed no irritative effect of this seasoning (data not shown).

The golden standard for diagnosis is the DB PCFC. Labial challenge has been suggested as an alternative (25, 26). However, it was not feasible for mustard because of the irritative effect.

It is difficult to obtain real blind PCFC to mustard. One study used flour in capsules, not exceeding the dose of 935 mg (7). Flour was selected for this study because it may not reflect the allergic risk of the seasoning. Moreover a metabisulfite-free mustard seasoning was used so as to avoid any adverse effect as a result of sulfite intolerance. The problem of taste was solved by four precautionary measures: very sweet, cold drink, taken immediately after mixing in the mustard and using a straw to reduce contact with the tongue. A pince-nez can be added. The amount ingested – 1340 mg – corresponds to a routine dose.

One subject (n°3) reacted to 40 mg, equivalent to 13.5 mg of mustard seeds, roughly equivalent to 0.8 mg of proteins (mustard seasoning is considered to contain 6% of proteins). This child, sensitized to mustard pollen (SPT 7 mm) and to rape pollen (SPT 3 mm), lives in the area of a mustard seasoning factory. An unpleasant smell arising from this factory is reported.

The existence of cross allergies between various plants of the Brassicaceae family has received little attention. The frequency of post-challenge rhinitis or conjunctivitis might indicate a previous cross-sensitization to pollens. No cross food allergy was observed in these 30 patients, but one case of allergy to rapeseed flour and to mustard was recently published (22). The vegetarian burgers currently on the increase in the US, and soon to be found in Europe, might well include rape proteins, so that this possibility has to be borne in mind.

Skin reactivity was observed with the two species of mustard: *B. nigra* and *B. juncea* pointing to cross-sensitization. *In vitro* cross-reactivity has been established for *S. alba* and *B. juncea* (21).

The frequency of mustard allergy is highly variable depending on the study: 3% according to Andre (11) and from 6 to 8.9% for Rance (12, 13). It represents only 1.1% of a series of 707 children presenting with food allergy, and <1% of the 196 adults with food allergy (10). This discrepancy might be explained by differences of consumption between south and east of France, concerning pickled onions and gherkins that contain mustard seeds and various spices.

A former study showed allergy to mustard in 42% of children sensitized to mustard (7). The rate is lower (23.3%) in this study. This discrepancy between sensitization and allergy is common for all foods. RAST and prick tests were not predictive of mustard allergy, whether carried out with native seeds, flour and seasoning or using an allergenic extract. The prescription of avoidance diets can only rely on a positive standardized challenge.

The seriousness of certain reactions argues for an informative labeling, the more so as it is often a masked allergen in many manufactured sauces.

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