

REGULAR ARTICLE

Sensitization and allergy to turnip rape: a comparison between the Finnish and French children with atopic dermatitis

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Keywords

Atopic dermatitis, Food allergy, Mustard, Oilseed rape, Turnip rape

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Received

1 March 2008; revised 18 June 2008;
accepted 11 August 2008.

DOI:10.1111/j.1651-2227.2008.01020.x

Abstract

Aim: Finnish children with atopic dermatitis (AD) are frequently sensitized and show positive food challenge to turnip rape. We examined whether French children are also allergic to this oilseed plant and whether mustard could be the cross-reacting allergen.

Methods: Turnip rape and mustard challenge was performed to 14 Finnish and 14 French children with atopic dermatitis and positive skin prick test to turnip rape. Specific IgE antibodies were measured by ImmunoCAP and enzyme-linked immunosorbent assay (ELISA).

Results: Open labial or oral challenge to turnip rape was positive in 14 (100%) Finnish and five (36%) French children and mustard challenge in five Finnish and five French children. IgE antibodies to oilseed rape and mustard were slightly more frequent in the Finnish (100% and 93%) than in the French (93% and 71%) children but rare (4%) in the 28 matched controls. The same findings were true for IgE antibodies to purified 2S albumin allergens, which showed similar cross-wise IgE inhibition patterns.

Conclusion: French children with atopic dermatitis show IgE antibodies to turnip rape, oilseed rape and mustard similarly to the Finnish children. 2S albumin allergens in the seeds of these plants are highly cross-reactive and therefore, they all could be important sensitizers in children with atopic dermatitis.

INTRODUCTION

In developed countries, food allergy is common in young children with atopic dermatitis (AD) (1). Cow's milk, egg, wheat, peanut, fish and soy are regarded as the main allergenic foods (2,3). Food consumption habits vary geographically causing differences in the prevalence of allergy to certain foods. New important food allergens may also appear in certain populations such as sesame seed allergy in Israeli and mustard allergy in French children (4–6). When we skin prick tested (SPT) mustard, we found a high sensitization rate in children with AD suspected for food allergy though they did not consume this spice. Therefore we included turnip rape (*Brassica rapa* ssp. *oleifera*) and oilseed rape (*B. napus* ssp. *oleifera*), which belong to the same *Brassicaceae* family as mustard (*Sinapis alba*) in the SPT series. A total of 1887 Finnish children were SPT with the seeds of turnip rape and oilseed rape and the sensitization rate was as high as 11%. Positive labial or oral challenges with turnip rape seeds were seen in 25 out of 28 (89%) tested children showing that turnip rape and closely related oilseed rape are new potential food allergens in children with AD (7). Seeds of turnip rape are increasingly used in vegetable oil production in Finland (8) and oilseed rape elsewhere in Europe but whether these oils contain relevant amounts of allergens like crude peanut oil (9) is at present not known.

Because the origin of the frequent sensitization to turnip rape in the Finnish children with AD remains unknown we

examined whether French children with AD would also be reactive to this oilseed plant. Turnip rape and the oilseed rape belong to the same *Brassicaceae* plant family as the yellow and oriental mustard, and the major allergens in the seeds of all four, Bra r 1, Bra n 1, Sin a 1, Bra j 1, are 2S storage albumins (10–12). Due to this we challenged the Finnish and French children with turnip rape and mustard and examined whether IgE antibodies from these children are cross-reactive to the purified 2S albumin allergens in the seeds of these plants.

MATERIALS AND METHODS

Patients

Fourteen Finnish children with AD were examined at the Department of Dermatology, Tampere University Hospital and 14 French children at the Department of Respiratory and Allergic diseases in Children and Adolescents, University Hospital in Toulouse. The children were collected randomly and inclusion criteria were AD and a positive SPT (wheal diameter ≥ 3 mm) to turnip rape. Table 1 shows associated atopic disorders and allergies in these children. All 14 Finnish children were allergic to wheat and egg, and 12 to cow's milk. Wheat and cow's milk allergies were confirmed by open food challenge. Thirteen French children were allergic to egg, eight to peanut and three to cow's milk. Ten Finnish children were sensitized to birch and eight French children to grass pollen. Twenty-eight age- and sex-matched

Table 1 Associated atopic disorders, food allergies and sensitization to pollens in turnip rape-sensitized Finnish and French children and in non-sensitized control children

	Finnish children N = 14	French children N = 14	Control children N = 28
Age, mean (range), years	3.8 (1.3–9.1)	4.7 (1.5–9.3)	4.2 (0.9–10.5)
Parents or siblings affected by atopic disorder, n (%)	14 (100)	10 (71)	22 (79)
Personal atopic disorder, n (%)			
Atopic dermatitis	14 (100)	14 (100)	28 (100)
Asthma	3 (21)	10 (71)	6 (21)
Allergic rhinitis	7 (50)	3 (21)	9 (32)
Food allergy, n (%)			
Egg	14 (100)	13 (93)	11 (39)
Milk	12 (86)	3 (21)	9 (32)
Wheat	14 (100)	1 (7)	11 (39)
Sensitization to inhalant allergens, n (%)			
Birch	10 (71)	Not tested	12 (43)
Grass pollen	3 (21)	8 (60)	5 (18)

Finnish AD children with negative SPT to turnip rape and oilseed rape served as controls in the IgE antibody tests (Table 1). These control children were selected randomly from the files by matching age and sex. The Ethical Committees of Tampere University Hospital and University Hospital of Toulouse approved the challenge study and informed consent was obtained from parents.

Labial and oral challenge with turnip rape and mustard

Turnip rape challenge was started by applying a small amount of oily mass of crushed seeds on the lip (7,13). The test was considered positive when wheals appeared on the lips or the surrounding skin, or when clear labial swelling was seen. If labial testing was negative, increasing doses of crushed seeds of turnip rape (10, 30, 100, 300 and 900 mg) mixed in an appropriate food were given orally every 20 min (7).

Mustard challenge was performed after a minimum of one week from the turnip rape challenge. Labial challenge was not performed due to the irritant effect of mustard (14). Increasing doses of crushed seeds of mustard (1, 5, 10, 20, 50, 100, 250 and 500 mg) mixed in an appropriate food were given orally every 20 min (15). All cutaneous, gastrointestinal and respiratory symptoms were registered during the challenges and 2 h thereafter. When a positive reaction appeared, the challenge was stopped and the child received appropriate medication.

Skin prick tests

SPTs were performed with crushed seeds of turnip rape (*Brassica rapa* ssp. *oleifera*), oilseed rape (*B. napus* ssp. *oleifera*) and mustard (*Sinapis alba*) moistened with physiological saline using a one-peak lancet (ALK-Abelló A/S, Hørsholm, Denmark) and the prick-prick test was performed as described previously (7). A mean wheal diameter ≥ 3 mm was considered positive when the negative control was 0. Histamine dihydrochloride (10 mg/mL, ALK-Abelló A/S, Hørsholm, Denmark) was used as a positive and saline

(Soluprick SQ, ALK-Abelló A/S, Hørsholm, Denmark) as a negative control.

IgE antibody measurements

Sera collected from all sensitized and control children were stored at -20°C until testing. IgE antibodies to oilseed rape and mustard were measured by ImmunoCAP (f316, f89, ImmunoCAP Specific IgE, Phadia AB, Uppsala, Sweden). Values ≥ 0.35 kU/L were considered positive. There is no commercial ImmunoCAP for turnip rape.

IgE antibodies to 2S albumins of turnip rape (Bra r 1), oilseed rape (Bra n 1) and mustard (Sin a 1) were examined by enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with 2S albumins purified from the seeds of these plants by gel filtration and cation exchange chromatography as described previously (10). Microtiter plates were coated with the allergen solutions $1\text{ }\mu\text{g/mL}$, $100\text{ }\mu\text{L/well}$ and after overnight incubation at 4°C post-coated for 1 h with 1% human serum albumin. After incubation with patient serum (diluted 1: 10) for 2 h, biotinylated anti-human-IgE (diluted 1: 1000, Vector Laboratories inc, Burlingame CA, USA) was added, followed by streptavidine-conjugated alkaline phosphatase (diluted 1: 3000, Bio-Rad Laboratories, Hercules CA, USA) and substrate (pNPP, Sigma-Aldrich Co, St Louis MO, USA). The colour reaction was measured at 405 nm with a Multiscan Ascent ELISA reader (Thermo Labsystems, Vantaa, Finland). Optical density values to 2S albumins of turnip rape, oilseed rape and mustard exceeding the highest absorbances in the 28 control children were considered positive.

ELISA inhibition experiments

To assess the IgE cross reactivity between the three allergens, inhibition experiments by ELISA were performed. Coating and post-coating were performed as described above, but the allergen volume added was $50\text{ }\mu\text{L}$ per well. Individual sera (diluted 1: 20) studied were first incubated for 1 h with an equal volume of the inhibitor and then put in the wells for 2 h. The inhibitors examined were 2S albumins of oilseed

Table 2 Challenge, skin prick test reactions and IgE antibodies to turnip rape, oilseed rape and mustard in turnip rape-sensitized Finnish and French children

Allergen	Finnish children N = 14 (%)	French children N = 14 (%)
Turnip rape and oilseed rape		
Positive challenge to turnip rape	14 (100)	5 (36)
Positive skin prick test		
to turnip rape	14 (100)	14 (100)
to oilseed rape	12 (86)	10 (71)
Positive IgE ImmunoCAP to oilseed rape	14 (100)	10 (71)
Positive 2S albumin IgE ELISA		
- to turnip rape	10 (71)	8 (57)
- to oilseed rape	10 (71)	8 (57)
Mustard		
Positive challenge	5 (36)	5 (36)
Positive skin prick test	14 (100)	14 (100)
Positive IgE ImmunoCAP	13 (93)	10 (71)
Positive 2S albumin IgE ELISA	9 (64)	7 (50)

rape, turnip rape and mustard tested at concentrations of 0, 0.1, 1, 10, 100 and 1000 ng/mL. After the incubations the experiments were continued as described for IgE ELISA.

Statistical analysis

Statistical comparison was made by using the Permutation and Fisher exact tests.

RESULTS

Challenge reactions

Turnip rape challenge was positive in 14 (100%) Finnish and five (36%) French children ($p < 0.001$, Table 2). Twelve Finnish and five French children showed positive labial challenge to turnip rape. Two Finnish children got facial urticaria in oral challenge to 40 mg and 140 mg of turnip rape. Oral mustard challenge was positive in five (36%) Finnish and five (36%) French children (Table 2). The five French children had negative turnip rape challenge. The mean dose of mustard causing positive challenge reactions was 442 mg

(range 16 to 936 mg) in the Finnish and 332 mg (range 86 to 436 mg) in the French children. Three Finnish children got facial urticaria, two abdominal pain and vomiting. Two of the French children got urticaria, one got rhinitis and asthma reaction requiring a bronchodilator. In addition two got abdominal pain and vomiting.

Skin prick test results

Due to the inclusion criterion, SPT to turnip rape was positive in all 14 Finnish (mean wheal diameter 6.5 mm) and 14 French (mean wheal diameter 7.6 mm) children (Table 2). SPT to oilseed rape was positive in 12 Finnish (mean wheal diameter 4.4 mm) and 10 French (mean wheal diameter 6.1 mm) children. SPT to mustard was positive in 14 Finnish (mean wheal diameter 5.7 mm) and 14 French (mean wheal diameter 7.1 mm) children. These SPTs were negative in all 28 control children.

IgE antibodies to oilseed rape and mustard

IgE ImmunoCAP to oilseed rape was positive in 14 (100%) Finnish and 10 (71%) French children (Table 2). The median IgE level to oilseed rape was 4.6 kU/L (IQR: 1.7, 29 kU/L) in the Finnish and 4.3 kU/L (IQR: <0.35, 13.3 kU/L) in the French children. IgE ImmunoCAP to mustard was positive in 13 (93%) Finnish and 10 (71%) French children (Table 2). The median IgE level to mustard was 3 kU/L (IQR: 1, 18.8 kU/L) in the Finnish and 3.6 kU/L (IQR: <0.35, 10 kU/L) in the French children. One control child had IgE antibodies to mustard (1.3 kU/L). The median total IgE level was 488 kU/L (IQR: 103, 4040 kU/L) in the Finnish, 1173 kU/L (IQR: 249, 2488 kU/L) in the French and 85 kU/L (IQR: 15, 835 kU/L) in the control children.

IgE antibodies to 2S albumins of turnip rape, oilseed rape and mustard

2S albumin IgE ELISA was positive to turnip rape in 10 Finnish and eight French children, to oilseed rape in 10 Finnish and eight French children and to mustard in nine Finnish and seven French children (Fig. 1, Table 2).

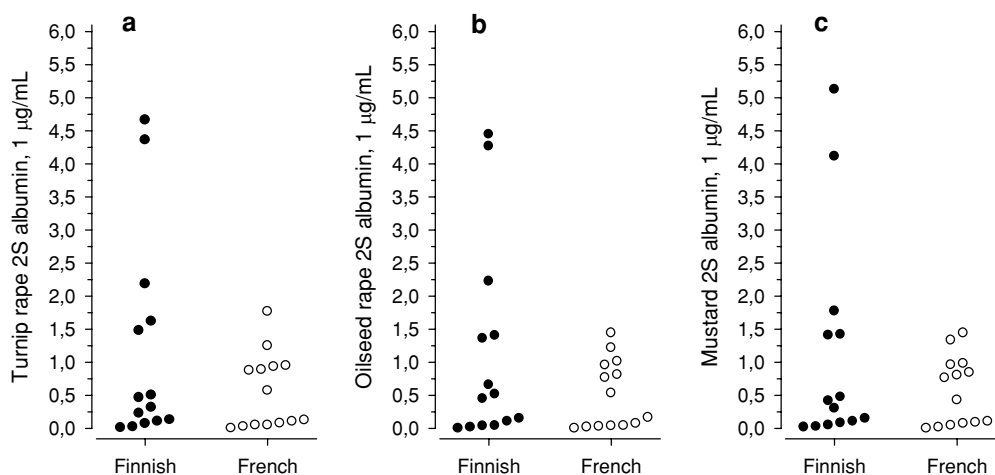


Figure 1 IgE antibodies by ELISA (absorbance 405 nm) to purified 2S albumins of turnip rape (A), oilseed rape (B) and mustard (C) in Finnish (N = 14) and French (N = 14) children.

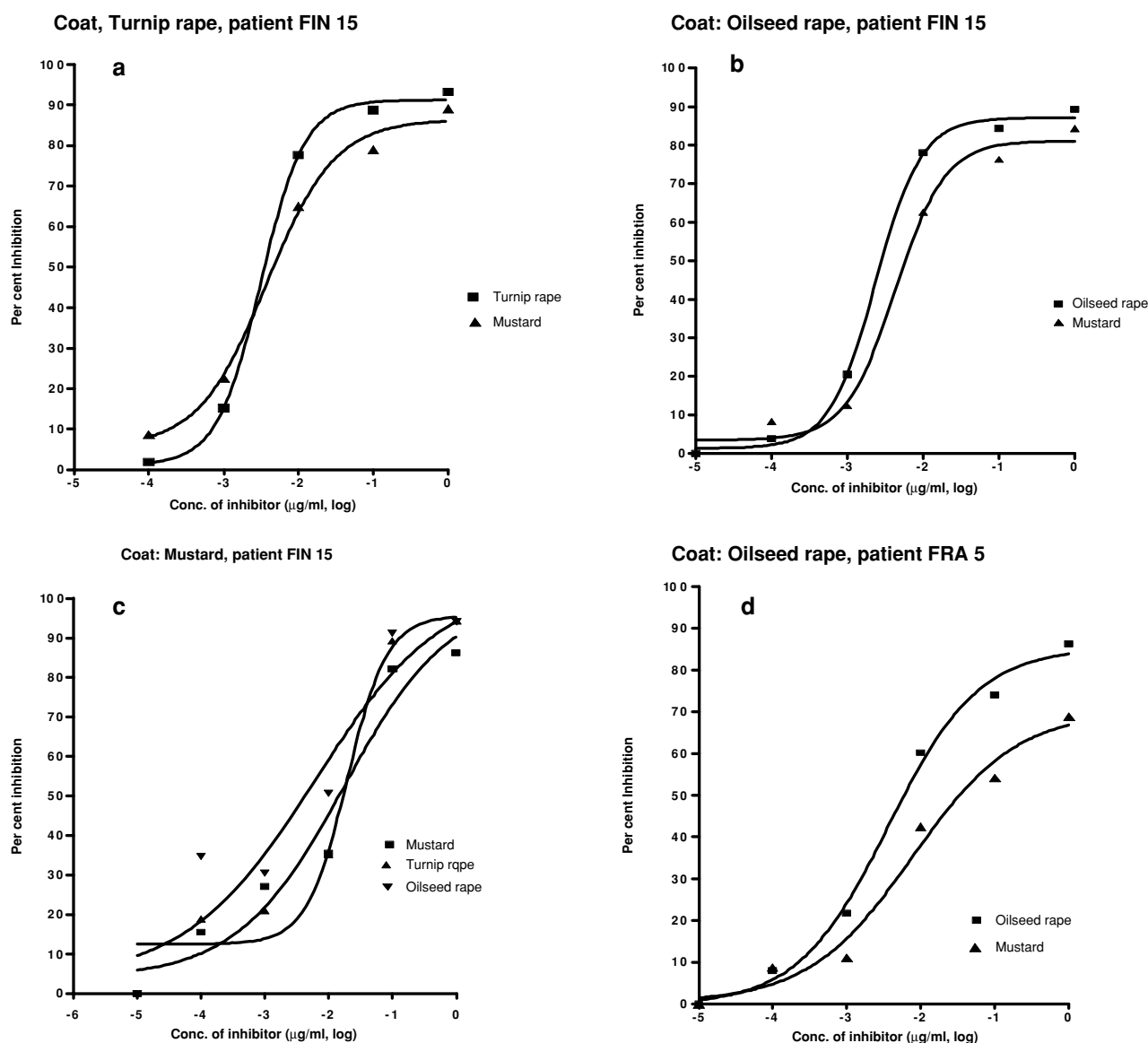


Figure 2 Examples of IgE-binding inhibition experiments with purified 2S albumins from turnip rape, oilseed rape and mustard by using IgE antibodies from the sera of a Finnish (FIN 15) and a French (FRA 5) child.

In the Finnish children the median IgE level to 2S albumins of turnip rape was 0.4 (IQR: 0.1, 1.8), to oilseed rape 0.5 (IQR: 0.04, 1.6) and to mustard 0.4 (IQR: 0.1, 1.5). In the French children the medians were 0.3 (IQR: 0.05, 0.9), 0.3 (IQR: 0.04, 1.0) and 0.3 (IQR: 0.05, 0.9), respectively. All three 2S albumin IgE levels were in the Finnish and French children significantly ($P < 0.001$) higher than those in the control children.

IgE inhibition experiments

Inhibition experiments were performed with all three 2S albumin allergens for sera from two Finnish and two French children with moderate to high IgE responses to all three allergens. Binding of IgE from the patient sera to solid-phase

allergens was effectively inhibited in a dose-dependent manner by all soluble inhibitors. Inhibition curves in Fig. 2, representing examples of one Finnish and one French serum, show that when either turnip rape or oilseed rape 2S albumins were on the solid-phase, the inhibitory capacities of the three 2S albumins were highly similar. When the plates were coated with mustard 2S albumins, somewhat more variation in the inhibition modes was seen, but due to the small number of experiments, no generalized conclusions could be drawn.

DISCUSSION

Previously we reported that 11% of the Finnish children with AD suspected for food allergy were sensitized to turnip rape

(7). In the present study we could show that also French children with AD were reactive in the skin prick testing and food challenge to the seeds of turnip rape. We also examined possible cross-reactivity between turnip rape, oilseed rape and mustard, all belonging to the *Brassicaceae* family. Ten (71%) of the turnip rape SPT positive French children showed positive SPT to the seeds of oilseed rape and all 14 (100%) to mustard. IgE ImmunoCAP was also frequently (71%) positive to oilseed rape and mustard. Finnish children exhibited similar high frequencies of positive test reactions to oilseed rape and mustard. Turnip rape challenge was positive in five (36%) French and all 14 (100%) Finnish children, but the frequency of positive mustard challenge was the same 36%. The difference in the turnip rape challenge results may be due to the fact that the Finnish children had somewhat slightly higher IgE levels to crude or 2S albumin allergens of turnip rape. On the other hand, the challenges were open and started with a non-validated labial challenge method. Mustard contains irritants such as capsaicin and isothiocyanates why labial challenge was not performed for mustard (14). To our knowledge, such irritants have not been described in the seeds of turnip rape. In agreement with this, turnip rape labial challenge did not cause positive reactions in any of 25 turnip rape SPT negative control children with AD in our previous study (7).

Sensitization to turnip rape has not been studied previously in France, but several studies have shown that mustard allergy is common there (5,6,15). This is not unexpected, because France is the largest consumer of mustard in Europe and previously it was added even to baby foods (14). In contrast to France, in Finland consumption of mustard is low and we are not aware of this spice being added to baby foods or consumed in any significant amounts by young children. It was therefore unexpected to find out that all Finnish children had positive SPT and IgE ImmunoCAP to mustard. In addition, five (36%) Finnish children showed positive mustard challenge, which is the same frequency as in the French children. The symptoms were urticaria, asthmatic reaction and abdominal complains, which are similar to those reported previously (5,15). The mean dose of mustard causing positive challenge reactions was somewhat higher in the Finnish (442 mg) than French children (332 mg). These doses are comparable to those reported, e.g. in soybean allergy in Europe (16). The mustard findings in the Finnish and French children could well be cross-reactions from the turnip rape sensitization or vice versa. Supporting this, the seeds of mustard, turnip rape and oilseed rape are known to contain highly homologous 2S albumins (10,11). We measured IgE antibodies to the purified 2S albumins of all these three seeds by ELISA and found that the median IgE levels to turnip rape, oilseed rape and mustard were about the same in the Finnish and French children. ELISA inhibition studies by IgE antibodies from the Finnish and French children confirmed that the 2S albumins of turnip rape, oilseed rape and mustard are highly cross-reactive. Sesame seeds also contain 2S albumins (17) but cross-reactivity seems unlikely, because only one of the present 14 Finnish children showed positive SPT to sesame seeds.

Food allergy is common in young children with AD and cow's milk, egg and wheat are regarded as major allergenic basic foods in the developed countries (1–3). In agreement with this, the present Finnish and French children with AD had frequently allergy to these foods but were also sensitized to pollens and had often allergic rhinitis or asthma. It seems unlikely that the multiple allergies or associated allergic disorders in the present Finnish and French children with AD could somehow predispose to the sensitization against the 2S albumin allergens in the seeds of turnip rape, oilseed rape and mustard because the non-sensitized control children had also rather high frequencies of various allergies and allergic disorders. Though the present series was rather small, the prevalence of wheat allergy was markedly higher in the Finnish than in the French children. This cereal seems to be rather common food allergen in the Finnish children with AD (18,19). In agreement with this, our previous case-control study of 64-turnip rape and oilseed rape sensitized Finnish children also found a significant association to wheat, but also to milk and egg sensitizations (20).

It is also of interest that the turnip rape and oilseed rape sensitized children in our previous study had been exclusively breast-fed for significantly longer period ($p < 0.05$) than the non-sensitized control children (20). In another study we found a few infants on exclusive breast-feeding who were already sensitized to turnip rape (10) suggesting that children could also become sensitized via breast-feeding. The possible source of sensitization could be rapeseed oil, which is manufactured from the oilseed rape and/or turnip rape. Rapeseed oil is increasingly used by the food industry in Europe and according to food labels it is added to margarine and even to baby foods including milk formulas. It is at present not known whether crude or refined rapeseed oil contain minute amounts of allergenic proteins, like peanut, sunflower and sesame oils do (9,21–23) and would thereby be capable of sensitizing children both in Finland and France. The importance of turnip rape and oilseed rape 2S albumins as sensitizing food allergens and their potential for cross-reactions seem obvious and therefore, it is of utmost importance to disclose the sensitization sources and routes in animal and laboratory experiments.

In conclusion, the present study showed that the young French children with AD, like the Finnish children, can be sensitized and reactive to turnip rape, oilseed rape and mustard. The 2S albumin allergens in the seeds of these plants were found to be highly cross-reactive and therefore, all three allergens could be important sensitizers in children with AD. When a child is sensitized to any of these allergens, avoidance of mustard can be recommended due to its potential to cause allergic reactions.

ACKNOWLEDGEMENTS

The authors thank MD Timo Palosuo for contributing to the ELISA experiments and valuable comments, MBc Hannu Kautiainen for statistical work, nurse Pirkko Burri for assistance with food challenges and Helena Honkasalo for

technical assistance. This study was supported by grants from the Tampere University Hospital Research Fund and the Finnish Society of Dermatology.

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