



SANNA POIKONEN

Turnip Rape and Oilseed Rape Allergy in Children
with Atopic Dermatitis



ACADEMIC DISSERTATION

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the Faculty of Medicine of the University of Tampere,
for public discussion in the Auditorium of Finn-Medi 1,
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UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION

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1. LIST OF ORIGINAL PUBLICATIONS

The thesis is based upon the following original papers, referred to in the text by Roman numerals (I-IV).

- I Poikonen S, Puumalainen TJ, Kautiainen H, Burri P, Palosuo T, Reunala T, Turjanmaa K. Turnip rape and oilseed rape are new potential food allergens in children with atopic dermatitis. *Allergy* 2006; 61:124-127.
- II Poikonen S, Puumalainen TJ, Kautiainen H, Palosuo T, Reunala T, Turjanmaa K. Sensitization to turnip rape and oilseed rape with atopic dermatitis: a case-control study. *Pediatr Allergy Immunol* in press. Jan 22, 2008 Epub ahead of print.
- III Poikonen S, Rancé F, Puumalainen TJ, Le Manach G, Reunala T, Turjanmaa K. Sensitization and allergy to turnip rape: a comparison between Finnish and French children with atopic dermatitis. Submitted.
- IV Puumalainen TJ, Poikonen S, Kotovuori A, Vaali K, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T. Napins, 2S albumins, are major allergens in oilseed rape and turnip rape. *J Allergy Clin Immunol* 2006; 117:426-32.

2. ABBREVIATIONS

AD	atopic dermatitis
APC	antigen-presenting cell
APT	atopy patch test
DC	dendritic cell
ELISA	enzyme-linked immunosorbent assay
GM-CSF	granulocyte magrophage colony-stimulating factor
IgA	immunoglobulin A
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
kDa	kilodalton
LTP	lipid transfer protein
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
M cell	microfold cell
mRNA	messenger ribonucleic acid
PBS	phosphate-buffered saline
PP	Peyer's patch
PR	pathogenesis related protein
PVDF	polyvinylidene difluoride
RAST	radioallergosorbent test
SCORAD	severity scoring of atopic dermatitis
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SPT	skin prick test
Th2	T helper lymphocyte type 2

3. ABSTRACT

Food allergy is common in young children with moderate to severe atopic dermatitis (AD). Cow's milk, egg, fish, soy, nuts and wheat account for most of the food allergies despite the enormous diversity of the diet. Sesame seed and mustard are new important food allergens. Seeds of turnip rape and oilseed rape are increasingly used for vegetable oil production, but whether these oilseed plants could be potential food allergens has not been studied.

In the first part of the present study (I), skin prick testing (SPT) showed a high (11%) sensitization rate to turnip rape and/or oilseed rape in 1887 Finnish infants and young children with AD examined for suspected food allergy. When 28 sensitized children were studied in more detail, most (89%) of them showed positive labial or oral challenge reactions to the seeds of turnip rape.

We next examined whether sensitization to turnip rape and oilseed rape could be associated with other food or pollen sensitizations. A case-control study (II) performed in 64 sensitized children (mean age 2.5 years) with AD showed significant ($p < 0.01$) sensitization rates to various foods (cow's milk, egg, wheat and mustard) and pollens (birch, timothy and mugwort).

Turnip rape and oilseed rape belong to the same *Brassicaceae* family as mustard which has been reported to cause food allergy in France. Due to this we examined children with AD and positive SPT to turnip rape (14 from France and 14 from Finland) and compared their reactivity to turnip rape, oilseed rape and mustard (III). Open labial or oral turnip rape challenges were positive in 36% of the French children and 100% of the Finnish children. Open oral challenges with mustard were positive in 36% of the French and the Finnish children. Moreover,

most of the French and Finnish children had positive SPTs and IgE antibodies in the serum both to oilseed rape and mustard.

In the last study (IV), IgE-binding allergens from oilseed rape and turnip rape seeds were purified and characterized by means of reversed-phase chromatography, N-terminal amino acid sequencing and mass spectrometry. The allergens were identified as 2S albumins, also known as napins. When the purified 2S albumins were compared to mustard 2S albumins, all determined N-terminal sequences of the large subunits were identical. Most of the 72 sensitized Finnish children with AD had IgE antibodies to 2S albumin allergens of turnip rape and oilseed rape in ELISA. The purified 2S albumins from turnip rape and oilseed rape caused positive SPT reactions in all 6 sensitized children with AD tested. In addition to having IgE antibodies to 2S albumins of turnip rape and oilseed rape, most of the sensitized 14 Finnish and 14 French children with AD showed IgE antibodies also to 2S albumins of mustard (III). Preliminary IgE inhibition experiments showed similar cross-wise inhibition patterns between the turnip rape, oilseed rape and mustard 2S albumin allergens.

In conclusion, the present study showed a high sensitization rate to turnip rape and oilseed rape in the Finnish infants and young children with AD. Positive labial and oral challenge reactions further substantiated that the oilseed plants could be clinically relevant food allergens especially in children with AD sensitized to multiple food and pollen allergens. The IgE-binding allergens were identified as 2S albumins which showed homology to mustard 2S albumins. Studies in the sensitized Finnish and French children with AD showed that mustard could be a cross-sensitizing allergen and also cause clinically relevant cross-reactions. Whether rapeseed oil added to food contains minute but sufficient amounts of 2S albumin allergens capable of sensitizing children and exacerbating AD similarly to other allergens needs to be examined in further studies.

TIIVISTELMÄ

Keskivaikeaa tai vaikeaa atooppista ihottumaa sairastavilla lapsilla ruoka-allergia on tavallista. Maito, muna, kala, soija, pähkinät ja vehnä aiheuttavat suurimman osan ruoka-allergioista. Seesamin siemen- ja sinappi-allergia ovat esimerkkejä uusista merkittävistä lasten ruoka-allergioista. Rypsin ja rapsin siemenistä valmistettua kasviöljyä käytetään enenevästi, mutta kasviöljyjen mahdollisuutta ruoka-allergian aiheuttajana ei ole tutkittu.

Tutkimuksen (I) osassa tehtiin ihopistokokeet rypsilille ja rapsilille 1887 atooppista ihottumaa sairastavalle lapselle ruoka-allergiaepäilyn vuoksi. Heistä 11%:lla ihopistokoe oli positiivinen rypsilille ja/tai rapsilille. Kaksikymmentäkahdeksan herkistynyttä lasta tutkittiin tarkemmin ja heistä suurimmalla osalla (89%) ruoka-ainealtistus rypsin siemenellä oli positiivinen joko huulialtistuksena tai syötynä.

Seuraavaksi tutkimme rypsi- ja rapsiherkistymiseen liittyviä muita ruoka- tai siitepölyherkistymiä. Tapaus-verrokkitutkimukseen (II) osallistui 64 atooppista ihottumaa sairastavaa rypsi- ja rapsiherkistynyttä lasta (keskiarvoikä 2.5 vuotta). Heillä rypsi- ja rapsiherkistymä liittyi merkitsevästi enemmän ($p < 0.01$) muihin ruoka-aine- (maito, muna, vehnä ja sinappi) ja siitepölyherkistymiin (koivu, timotei ja pujo) kontroleihin verrattuna.

Rypsi ja rapsi kuuluvat samaan *Brassicaceae* – kasviheimoon kuin sinappi, joka on yleinen ruoka-allergeeni ranskalaisilla lapsilla. Työssä (III) tutkimme rypsi-, rapsi- ja sinappiherkistymän suhteen 14 ranskalais- ja 14 suomalaislasta, joilla kaikilla oli atooppinen ihottuma ja positiivinen ihopistokoe rypsilille. Ruoka-ainealtistus rypsilä oli positiivinen 36% ranskalaisista lapsista, kaikilla suomalaisilla lapsilla ja sinappialtistus oli positiivinen 36% sekä ranskalais- että

suomalaislapsista. Suurimmalla osalla ranskalaisista ja suomalaisista lapsista ihopistokoe ja seerumin IgE vasta-aine rapsille sekä sinapille olivat positiivisia.

Viimeisessä (IV) työssä rypsin ja rapsin IgE:tä sitovat proteiinit puhdistettiin ja identifioitiin käänteisfaasikromatografian, aminohapposekvensoinnin ja massaspektrometrian avulla. Potilaiden IgE sitoutui pääasiallisesti proteiineihin, jotka osoittautuivat 2S albumiineiksi (napiineiksi). Kun sinapin 2S albumiineja verrattiin, olivat ne rypsin ja rapsin 2S albumiinien N-terminaalisekvenssien kanssa samanlaisia. Suurimmalla osalla 72 testatusta atooppista ihottumaa sairastavasta suomalaisesta lapsesta oli IgE vasta-aineita sekä rypsin että rapsin 2S albumiineille ELISA:ssa. Kuudelle herkistyneelle, atooppista ihottumaa sairastavalle lapselle tehtiin ihopistokokeet puhdistetuilla rypsin ja rapsin 2S albumiineilla. Ihopistokokeet olivat positiivisia kaikilla 6 potilaalla. Rypsi ja rapsin 2S albumiinien lisäksi suurimmalla osalla atooppista ihottumaa sairastavista suomalaisista ja ranskalaisista lapsista oli myös IgE vasta-aineita sinapille (III). Alustavissa IgE inhibiitiokokeissa voitiin osoittaa samankaltaisia inhibiitiökäyriä kaikilla kolmella 2S albumiinilla.

Atooppista ihottumaa sairastavilla suomalaisilla pikkulapsilla rypsi- ja rapsiherkistymä on tavallista. Positiiviset tulokset rypsi-altistuksessa osoittivat, että rypsi ja rapsi voivat olla kliinisesti merkityksellisiä ruoka-allergeeneja erityisesti atooppista ihottumaa sairastavilla, muille ruoka-aineille ja siitepölyille herkistyneillä lapsilla. Rypsin ja rapsin allergeeneiksi osoittautuivat 2S albumiinit, jotka ovat homologisia sinapin 2S albumiinien kanssa. Tutkimukset osoittivat edelleen, että atooppista ihottumaa sairastavat sinapille herkistyneet ranskalaiset lapset voivat olla suomalaisten lasten tavoin herkistyneitä myös rypsillem ja rapsille. Jatkossa täytyy selvittää voiko rypsiöljyissä olla pieniä määriä rypsin ja rapsin 2S albumiineja, jotka riittäisivät herkistämään atooppista ihottumaa sairastavia lapsia ja aiheuttamaan heille atooppisen ihottuman pahenemisen.

4. INTRODUCTION

The prevalence of food allergy in children ranges from 2.3 to 8% (Bock 1987, Pereira et al. 2005). In children with moderate to severe atopic dermatitis (AD), the prevalence of food allergy is as high as 37-90% (Eigenmann et al. 1998, Sampson 1992, Hill et al. 2007). Relatively few foods account for the majority of food allergies despite the diversity of diet. Cow's milk, egg, fish, soy, wheat and peanut account for about 90% of reported food allergies in infants and young children (Sampson and McCaskill 1985, Burks et al. 1998). The order of importance of food allergens varies in different countries, reflecting a possible interaction of genetic risk factors, environmental factors, as well as cultural and dietary habits.

Mustard has been shown to be one of the four most prevalent food allergens in French children after egg, peanut and milk (Rancé et al. 1999a). This prompted us to include mustard in our SPT series at Tampere University Hospital for children suspected for food allergy. As the rate of sensitization was high, we included turnip rape (*Brassica rapa* ssp. *oleifera*) and oilseed rape (*Brassica napus* ssp. *oleifera*) in the SPT series. These two oilseed plants belong to the same *Brassicaceae* family as mustards and we found again a constant high number of positive SPT reactions. Oilseed rape is the most commonly cultivated oilseed crop in Europe and turnip rape is especially used by the Finnish food industry, but food allergies to turnip rape or oilseed rape have not been investigated before. Previous studies concerning possible allergenic properties of oilseed rape and turnip rape have focused on the pollens on these plants. Allergy to pollen of oilseed rape is rare and data on turnip rape pollen are limited (Fell et al. 1992, Toriyama et al. 1995).

This study aimed to investigate the prevalence of sensitization to turnip rape and oilseed rape in children with AD and to perform turnip rape and mustard food challenges in the sensitized children to examine their clinical reactivity to the seeds of these plants. Further aims were to examine whether sensitization to turnip rape and oilseed rape is associated with other food allergies. In addition, the study aimed to characterize the major allergens in turnip rape and oilseed rape.

5. REVIEW OF THE LITERATURE

5.1 Atopy and allergic diseases

Allergy is defined as a hypersensitivity reaction initiated by immunologic mechanisms; it can be either antibody or cell mediated (Johansson et al. 2001). Most patients suffer from immunoglobulin E (IgE) mediated allergy, also referred to as type I allergy. Non-IgE mediated allergy includes type IV allergy mediated by antigen-specific T cells (e.g. allergic contact dermatitis) or it can be triggered by immunoglobulin G (IgG) isotype as in the classical, but now rare, serum sickness (Coombs et al. 1964). Atopy is a tendency to produce IgE antibodies in response to environmental allergens and to develop typical allergic symptoms (Coca et al. 1923, Johansson et al. 2001, Darsow et al. 2005). Manifestations of atopic diseases encompass allergic rhinitis, conjunctivitis, asthma, anaphylaxis, AD and urticaria. Food allergy shows a wide clinical picture involving the skin and the gastrointestinal and respiratory tracts (Johansson et al. 2001, Darsow et al. 2005).

5.2 Atopic dermatitis

5.2.1 Epidemiology and genetics

Atopic dermatitis (AD) is a pruritic chronic inflammatory skin disease that commonly presents during early infancy and childhood, but can persist or start in adulthood (Hanifin and Rajka 1980, Leung and Bieber 2003). The diagnosis is made clinically and several diagnostic criteria have been developed (Hanifin and Rajka 1980, Darsow et al. 2005, Williams 2005). The commonly used Hanifin and Rajka criteria state three of four main symptoms to be fulfilled; pruritus, typical morphology and distribution, chronic or chronically relapsing course, and atopic personal or family history, in addition to three minor criteria among a list

of 21 (Hanifin and Rajka 1980). The localisation of AD varies at different ages, but the clinical appearance of the skin lesions remains the same (Spergel and Paller 2003, Akdis et al. 2006). The European Task Force on Atopic Dermatitis has developed the SCORAD (SCORing AD) index to create a consensus on assessment methods in AD (Severity scoring of atopic dermatitis 1993). The index is made by combining extent, severity and subjective symptoms of AD. An index result <25 indicates mild, 25-50 moderate and ≥ 50 severe form of AD (Dermatology 1993).

In developed countries the prevalence of AD has been 10-20% in children (Leung and Bieber 2003, Asher et al. 2006). The prevalence has increased two- to threefold during the past decades and the disease seems to be more common in higher social groups (Taylor et al. 1984). The course of AD in childhood is variable. In one study, 20% of the children with onset of AD before two years of age had persisting symptoms and 40% had intermittent symptoms by the age of seven years (Illi et al. 2004). When the onset of AD is after childhood, in most cases it occurs between the ages of 18 and 29 years (Ozkaya 2005). In a population-based study involving 12 different countries the prevalence of AD was 7.1% in adults, ranging from 2.2% in Switzerland to 17.6% in Estonia (Harrop et al. 2007).

A recent worldwide study on the occurrence of atopic disorders in childhood showed a strong correlation in a given country between the prevalence of AD, asthma and allergic rhinoconjunctivitis (Asher et al. 2006). When affected children are followed up, AD is often the first step in the atopic march towards asthma and allergic rhinitis. The German Multicenter Atopy Study including 1314 children showed that 68% of infants who had AD by three months of age were sensitized to aeroallergens by 5 years of age and the risk increased with a positive family history of atopic diseases (Bergmann et al. 1998).

AD is a multifactorial disorder caused by the combined influence of genetic and environmental factors. Previous genetic studies have focused on immunological pathophysiology and disclosed several possible candidate genes, such as those on

chromosome 5q31-33 which contains a cluster of Th2 cytokine (IL 3, 4, 5, 13) genes (Forrest et al. 1999). AD has also been linked to markers at chromosome 11q13, including the gene encoding for the β chain of the high-affinity receptor for IgE (Cox et al. 1998). Detection of filaggrin as a major gene for AD was a breakthrough and recent studies underline the importance of a genetically determined epidermal barrier disruption in AD (Palmer et al. 2006). Filaggrin is a key protein that facilitates terminal differentiation of the epidermis and formation of the skin barrier. Two independent loss-of-function genetic variants in the gene encoding filaggrin are very strong predisposing factors (odds ratio 4.09) for atopic dermatitis. These variants are carried by approximately 9% of people of European origin and also show highly significant association with asthma occurring in the context of AD (Baurecht et al. 2007).

5.2.2 Immunopathogenesis

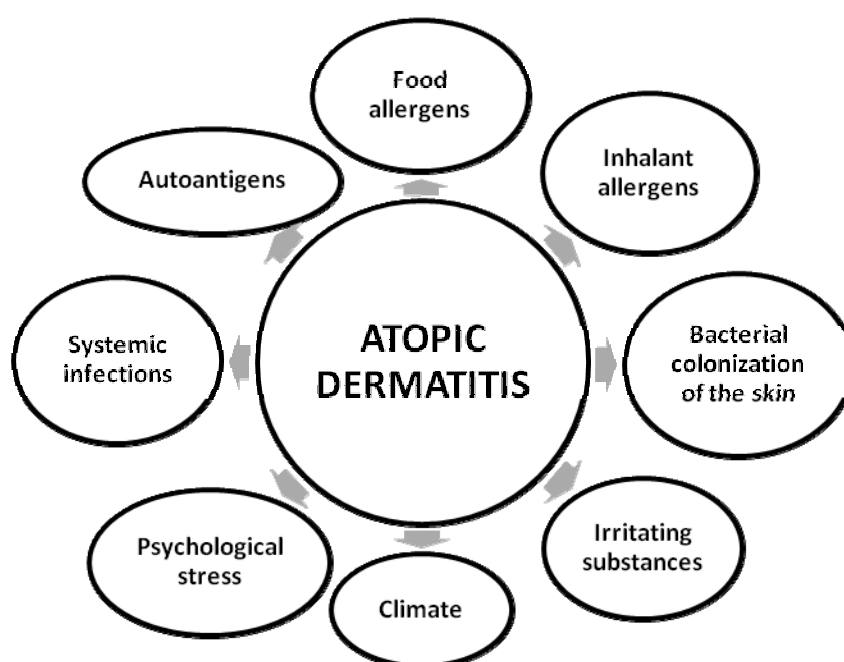
Interactions between susceptibility genes, the host's environment and immunological factors contribute to the pathophysiology of AD (Novak et al. 2003). Acute eczematous lesions are characterized by a marked epidermal intercellular oedema and striking infiltration of CD4⁺ T cells. Chronic lichenified lesions are characterized by an acanthotic epidermis and parakeratosis. Macrophages dominate the dermal mononuclear cell infiltrate which also contains eosinophils. The cytokine expression pattern is markedly different between acute and chronic skin lesions in AD. Acute lesions show an increased number of Th2 cells expressing mRNA of the interleukins IL-4 and IL-13. By contrast, chronic skin lesions have significantly fewer cells expressing mRNA of IL-4 and IL-13, but increased numbers of cells expressing mRNA of IL-5, GM-CSF, IL-12, and interferon γ (Leung and Bieber 2003, Bieber 2008).

Epidermal keratinocytes seem also to be involved and it is of interest that mechanical trauma, such as scratching, releases tumour necrosis factor α and many other proinflammatory cytokines from these cells. Moreover, keratinocytes are also an important source of thymic stromal lymphopoietin, which activates dendritic cells to prime naive Th cells to produce IL-4 and IL-13 (Soumelis et al. 2002). These findings might explain the link between scratching and the

triggering of Th2-mediated skin inflammation in AD. Recently, a novel Th2-derived cytokine, IL-31, known to cause severe pruritus and eczema in animal models, was identified also in the skin of patients with AD (Sonkoly et al. 2006).

Increased numbers of *S. aureus* are found in over 90% of AD skin lesions and this microbe is known to trigger the AD (Leung 2003, Bieber 2008). Exacerbation of AD by this microbe seems to be mediated by superantigens which can activate T cells and especially IL-31 production in skin-homing Th2 cells (Sonkoly et al. 2006). Moreover, most patients with AD produce IgE antibodies against staphylococcal superantigens and their levels correlate with the severity of the disease. AD skin is also deficient in the antimicrobial peptides needed for host defense against bacteria and viruses. Thus, inadequate defence of the host allows *S. aureus* to colonise in AD skin and produce superantigens which then may cause inflammation by several mechanisms (Howell et al 2006). In addition, there is evidence that the opportunistic yeast *Malassezia* species represents a contributing factor in adults with AD (Faergemann 1999, Scheynius et al. 2002).

Figure 1. Trigger factors for atopic dermatitis. Adapted from Werfel and Breuer (2004).



5.2.3 Allergens and atopic dermatitis

AD skin contains an increased number of IgE-bearing Langerhans cells and inflammatory dendritic epidermal cells expressing the high-affinity receptor for IgE (Leung and Bieber 2003). These antigen-presenting cells seem to have an important role in allergen presentation to T cells. They can migrate to the lymph nodes and stimulate naive T cells. In addition, Langerhans cells with high-affinity receptors for IgE need to be present to provoke eczematous skin lesions by application of aeroallergens on the skin (Langeveld-Wildschut et al. 2000). The isolation of T cells from AD skin lesions and allergen patch test sites that selectively respond to house-dust mite or birch pollen allergens provides further evidence that aeroallergens could elicit cell-mediated eczematous reactions in the skin of patients with AD (van Reijssen et al. 1992, Langeveld-Wildschut et al. 2000).

Common or pollen-related food allergens can be triggering factors in childhood AD (Breuer et al. 2004a, Breuer et al. 2004b, Darsow et al. 2004). AD in children is associated with sensitization especially to egg, cow's milk, wheat, peanut and soy (Illi et al. 2004, Peroni et al. 2007, Hill et al. 2008). Placebo-controlled food challenges in sensitized infants and young children have confirmed that these foods induce frequently flares of AD (Niggemann et al. 1999, Breuer et al. 2004b, Werfel and Breuer 2004). In older children, adolescents and adults, pollen-related foods can provoke flares of AD (Reekers et al. 1999, Breuer et al. 2004a). Moreover, application of food allergens by an atopy patch test on the skin of sensitized children elicits eczematous reactions similarly to aeroallergens in adults (Isolaure and Turjanmaa 1996, Turjanmaa et al. 2006). Importantly, T cells specific to food allergens have been cloned from the skin lesions of patients with AD, providing direct evidence that also foods could contribute to eczematous reactions in AD skin (van Reijssen et al. 1998).

5.2.4 Treatment of atopic dermatitis

The treatment of AD consists of a wide variety of therapies ranging from emollients to topical and systemic drugs, from identification and elimination of allergens to immune therapy, and finally from caring for emotional factors to

environmental factors. Treatments should be individually tailored and dependent on the age of the patient and the severity of the AD.

The genetically disturbed function of the skin barrier in AD results in dry skin (xerosis) and increased transepidermal water loss. Irritants such as soaps or detergents and abrasive clothing can worsen the skin condition. Soaps with a neutral pH and low defatting activity are preferred. The regular use of emollients is generally recommended and together with skin hydration it represents the mainstay of the general management of AD (Mc Henry et al. 1995, Lodén 2005, Williams 2005). Topical glucocorticoids are routinely used to control acute exacerbation of AD (Leung and Bieber 2003, Williams 2005, Akdis et al. 2006). Mild glucocorticoids such as hydrocortisone are usually sufficient for infants and young children with AD. More potent glucocorticoids are needed for adolescents and adult patients. Topical glucocorticoids have a risk for local adverse effects which is directly related to their potency and length of use (Williams 2005). Besides an anti-inflammatory effect, topical glucocorticoids contribute to a reduction of skin colonization by *S. aureus* (Akdis et al. 2006). Topical antimicrobial therapy, such as chlorhexidine and fucidic acid, can be used for localized infected lesions and systemic first- or second-generation cephalosporins for extensive superinfection with *S. aureus* (Akdis et al. 2006).

The calcineurin inhibitors pimecrolimus and tacrolimus are novel topical treatments for AD. Local burning sensation of the skin has been the only common side effect (Leung and Bieber 2003). In the EU, pimecrolimus cream (1%) and tacrolimus ointment (0.03%) are approved for the treatment of AD in children aged 2 years and older. Tacrolimus ointment (0.1%) is only approved for use in adults.

Natural sunlight is frequently beneficial for patients with AD and heliotherapy in the south during winter improves AD (Autio et al. 2002). A course of narrow-band ultraviolet B (311 nm) is at present a widely used and efficient way to treat older children and adult patients (Reynold et al. 2001).

Systemic treatment of AD includes antihistamines such as sedating hydroxyzine used at bedtime to control pruritus. A short course of oral corticosteroid is sometimes needed to control acute exacerbations in adolescents and adults. Use of systemic corticosteroids in children should be avoided. Severe AD refractory to conventional therapy may respond to ciclosporin or azathioprine (Leung and Bieber 2003). A recent study investigated the efficacy of an allergen-specific immunotherapy in patients with AD sensitized to house dust mite allergens with promising results (Werfel et al. 2006). However, further controlled studies are needed to determine the future role of immunotherapy with aero- or other allergens for AD (Akdis et al. 2006). Unlike allergic rhinitis and asthma, anti-IgE treatment has not been proven to be effective in AD. Confirmation of the efficacy of pre- and probiotics in the prophylaxis and treatment of childhood AD requires well-controlled prospective studies (Lee et al. 2008).

5.3 Food allergy

Food allergy is an adverse immunological reaction with clinical symptoms to food and it can be either IgE-mediated or non-IgE-mediated (Sicherer 2002). For practical reasons, food allergens are often divided into two classes: one group is exacerbated by so-called nutritionally central foods, the other by pollen-related foods. The nutritionally central food allergens include cow's milk, egg, fish, soy, wheat and peanut. These foods account for about 90% of reported food allergies in infants and young children (Burks et al. 1998, Sicherer 2002). Birch pollen-allergic individuals are known to react to raw fruits and vegetables. One of the manifestations is AD (Breuer et al. 2004a).

5.3.1 Prevalence

The prevalence of self-reported food hypersensitivity was 12% in 17,280 adults in the European Community Respiratory Health Survey performed in 15 different countries. It varied from 4.6% (Spain) to 19.1% (Australia) (Woods et al. 2001). An epidemiologic study involving 33,110 persons in France gave a prevalence of 3.2% for self-reported food hypersensitivity. In children under 3 years of age the prevalence was 4.1% and 2.8% in children 3 to 6 years of age

(Kanny et al. 2001). There is a strong heterogeneity in the prevalence of food allergy in different studies. The explanation for that could be differences in study design, methodology and populations. The prevalence of self-reported food allergy is much higher compared with objective evaluations (Rona et al. 2007). In unselected paediatric population studies food challenges have shown that the prevalence of food allergy ranges from 2.3 to 8% (Bock 1987, Roehr et al. 2004, Zuberbier et al. 2004, Pereira et al. 2005). A birth cohort of 969 infants revealed challenge-proven food allergy in 2.2 to 5.5% of infants during the first year of life (Venter et al. 2006).

The prevalence of food allergy in children with AD varies with the age of the patient and severity of AD. In children with moderate to severe AD, the prevalence of IgE-mediated food allergy has been 37-90% (Eigenmann et al. 1998, Sampson 1992, Hill et al. 2007). If the severe AD has started within the first three months of life, the frequency of high-risk IgE levels to milk, egg and/or peanut has been shown to be as high as 64% (Hill et al. 2008).

Relatively few foods have been held responsible for most food allergies despite the enormous diversity of the diet. Cow's milk, egg, fish, soy, wheat and peanut account for about 90% of reported food allergies in infants and young children (Burks et al. 1998, Rancé 1999a). The order of importance of food allergens varies in different countries, reflecting a possible interaction of genetic risk factors, environmental factors, cultural and dietary habits. For example, in the United States the most common food allergens in children are cow's milk, egg, peanut, wheat, soy, tree nuts, fish and shellfish (Sampson and McCaskill 1985, Burks et al. 1998), in France egg, peanut, cow's milk, mustard, fish, hazelnut, kiwi and wheat (Rancé et al. 1999b) and in Israel egg, cow's milk, sesame seed, peanut, soy, nuts and strawberry (Dalal et al. 2002). In population-based studies of cow's milk allergy confirmed by food challenge the incidences were 2% to 2.8% in children followed from birth to one year of age (Høst and Halken 1990, Saarinen et al. 2000, Schrandt et al. 1993, Venter et al. 2006). The point prevalence of allergy to egg has been reported to be 1.3% in children at one year and 1.6% at 2.5 years of age (Eggesbø et al. 2001, Venter et al. 2006). Early

childhood allergies to milk, egg, soy and wheat usually resolve by school age (Sampson and McCaskill 1985, Sampson and Scanlon 1989, Bishop et al. 1990, Høst and Halken 1990, Saarinen et al. 2005, Savage et al. 2007, Skripak et al. 2007). Peanut, tree nut and seafood allergies are generally considered permanent, and therefore adults are often still allergic to these foods. One study, however, has shown that peanut allergy is outgrown in about 21.5% of patients (Skolnick et al. 2001). Allergies to fruits and vegetables are common in older children and adults especially sensitized to pollens (Eriksson et al. 1982, Ortolani et al. 1988, Bircher et al. 1994).

5.3.2 Clinical manifestations

Food-induced allergic reactions show a wide clinical picture involving the skin, the gastrointestinal and respiratory tracts, and in severe anaphylactic cases the cardiovascular system. The diverse clinical manifestations may be localized to the site of allergen contact or they may be systemic, occurring in different organs (Sicherer and Sampson 2006).

Skin manifestations are frequent and IgE-mediated symptoms include urticaria and angioedema. Acute urticaria may be local due to contact with food proteins or generalized. The delayed skin symptoms manifest as a flareup of AD (Niggemann et al. 2001, Sampson 2003). Common food allergens, pollen-related foods and inhaled allergens may be trigger factors for persistent moderate to severe AD (Breuer et al. 2004a, Breuer et al. 2004b, Darsow et al. 2004). Placebo-controlled food challenge studies with milk, egg, wheat and soy have shown that food allergens can induce flareups of AD in a subset of sensitized infants and children (Niggemann et al. 1999, Breuer et al. 2004b). In addition, the pollen-related foods may provoke flareups of AD in sensitized older children, adolescent and adults (Reekers et al. 1999, Breuer et al. 2004a, Werfel et al. 2007). Non-IgE mediated skin symptoms include food-induced protein contact dermatitis especially among food handlers (Hjorth and Roed-Petersen 1976).

Food-induced respiratory symptoms mediated by IgE are wheezing, cough, asthma and rhinoconjunctivitis (Sicherer 2002, Sampson 2003). Exposure takes place through ingestion, but in some cases, inhalation of airborne food particles

may trigger respiratory reactions (James and Crespo 2007). Isolated or chronic asthma and rhinitis induced by food are rare. Food-induced asthma is more common in young children than in older children and adults. The role of food allergy in otitis media is controversial (James 2003).

Manifestations of food allergies in the gastrointestinal tract typically cause nausea, abdominal pain, vomiting, constipation, diarrhea and loss of weight (Iacono et al. 1995, Sicherer and Sampson 2006, Bischoff and Crowe 2005). Eosinophilic esophagitis, gastroesophageal reflux, dietary protein proctitis, enterocolitis and enteropathy are also rare manifestations of gastrointestinal food allergies (Iacono et al. 1996, Spergel et al. 2002, Bischoff and Crowe 2005)

In pollen-food allergy syndrome the symptoms are IgE-mediated and frequently localized to the oropharynx, including pruritus and angioedema of the lips, surrounding skin, palate, tongue or throat. Occasionally it may cause a sensation of tightness in the throat and even systemic symptoms like anaphylactic shock (Kelso 2000, Sicherer 2001, Ferreira et al. 2004).

Anaphylaxis is a generalized, potentially life-threatening hypersensitivity reaction. This reaction often develops gradually, usually starting with itching of the oropharynx, the palms, or soles, and with local urticaria. It develops to a multiple organ manifestation and culminates in hypotension and shock (Sampson et al. 2006, Simons 2007). Food-associated exercise-induced anaphylaxis is a subset of anaphylaxis. In this severe form of allergy, food triggers anaphylaxis only if the food is ingested before physical exercise. Wheat is the most common cause in exercise-induced anaphylaxis (Varjonen et al. 1997, Palosuo et al. 1999, Sicherer 2002).

5.3.3 Diagnosis

The evaluation of food allergy is based on a detailed clinical history and physical examination. Confirmation of a food allergy diagnosis rests on determination of food-specific IgE antibodies and SPTs, the results of elimination diets, and responses to oral food challenges (Sicherer and Sampson 2006).

Oral food challenge

The double-blind, placebo-controlled oral food challenge represents the gold standard for the diagnosis of food allergy. Standardization of food challenges in patients with immediate reactions has recently been described in a position paper by the European Academy of Allergology and Clinical Immunology Subcommittee. The paper proposes that an open oral food challenge controlled by a physician is sufficient for a diagnosis of food allergy in infants and children ≤ 3 years of age (Bindsvlev-Jensen et al. 2004). The patient eats gradually increasing amounts of the challenge food under observation by a physician. An alternative, or a first step to the oral challenge, could be a labial food challenge. It is simple, rapid to perform, and associated with only a low risk of systemic reaction (Rancé and Dutau 1997). It has not yet been validated, however, against oral food challenge. In addition to immediate reactions, patients with AD often present delayed reactions to an oral food challenge. Breuer et al. (2004b) observed that more than 50% of all positive oral challenges in children with AD were associated with an exacerbation of eczema and isolated eczematous reactions were seen in 12% of all positive challenges. The suspected food should be eliminated over a period of some weeks, e.g. 4-6 weeks, and if the symptoms improve, the oral food challenge should be performed. The skin must be scored by SCORAD before the challenge and at least after 24 h. A difference of at least 10 SCORAD points is usually considered a positive reaction. If the challenge is negative, the suspected food should ideally be administered over a period of several days (Werfel et al. 2007). Two studies found that 10% - 25% of positive double-blind, placebo-controlled oral food challenges were not IgE-mediated (Niggemann et al. 2001, Breuer et al. 2004b). Therefore a suspicion of food allergy rather than proof of specific IgE should be the indication to perform a food challenge especially in children with moderate to severe AD. Oral challenges are also needed to evaluate the resolution of the allergy.

Skin prick tests

Skin prick tests (SPT) are commonly used to diagnose food-specific IgE sensitization. A commercial one-peak lancet is pressed through a drop of an allergen extract into the epidermis on the volar side of the forearm (Høst et al.

2003). The prick-prick method (lancet is pricked into the fruit and immediately thereafter into the skin) is used when evaluating allergy to fresh fruits and vegetables because of the allergen lability of the commercially prepared food extracts (Dreborg and Foucard 1983). In the presence of food-specific IgE, the allergen interacts with the IgE on the surface of cutaneous mast cells. If antibodies are present, mast cells degranulate and release mediators that cause a localised wheal and flare reaction within 15 minutes. Positive (histamine dihydrochloride) and negative (saline) controls are used to prove that the immune response is not blocked and to rule out dermographism, a response to local trauma that causes the same symptoms as a positive reaction. The SPT is positive if the mean wheal diameter is at least 3 mm or greater and the negative control is zero (Sicherer 2002, Høst et al. 2003). The diagnostic sensitivity and specificity varies between different foods, techniques, reading systems and age groups (Akdis et al. 2006). The sizes of SPT reactions to egg, milk and peanut have been studied in order to determine cut-off levels of SPT in children for discrimination between true allergy and sensitization only. So far the levels vary between the same allergens in different studies (Sporik et al. 2000, Hill et al. 2004, Verstege et al. 2005, Knight et al. 2006). In one study, negative SPT results confirmed the absence of IgE-mediated allergy and the predictive accuracy was over 95% (Sampson and Albergo 1984).

IgE antibody measurements

A serum test to determine IgE antibodies to foods (eg, radioallergosorbent test, RAST previously, ImmunoCAP, Phadia Ab, Uppsala, Sweden) is another way to evaluate IgE-mediated food allergy. The allergen is bound to a solid matrix and exposed to the patient's serum. IgE antibody from the patient's serum binds to the protein matrix and is detected by use of a second labelled antibody specific for IgE (Yunginger et al. 2000). Findings of studies with children support the view that higher concentrations of food-specific IgE correlate with a higher likelihood of clinical reaction. Positive predictive values of 90% and 95% have been determined to egg, milk, peanut and fish in order to make oral food challenge unnecessary in selected children. As yet, no uniform decision values are available (Sampson and Ho 1997, Sampson 2001, Celik-Bilgili et al. 2005).

No decision points for specific IgE levels have been established for eczematous reactions to foods.

Atopy patch test

The atopy patch test is an epicutaneous test with aeroallergens and foods (cow's milk, egg, cereals and peanut) that may help to identify food allergy in patients with AD (Turjanmaa et al. 2006). It is performed on the skin of the back with a technique similar to the conventional patch test for the diagnosis of classical contact allergy. The occlusion time is 48 h and the readings are at 48 h and 72 h. In different studies the sensitivity varied from 0.18 to 0.89 and specificity from 0.35 to 0.97 (Kekki et al. 1997, Majamaa et al. 1999, Vanto et al. 1999, Roehr et al. 2001, Strömberg 2002). This may be explained by differences in the foods, methods and study populations. Combining the APT with SPT or specific IgE, measurement gave improved sensitivity and specificity in the diagnosis of milk, egg, wheat and soy allergy in a study of 98 children, but there was no significant reduction of need for food challenges (Roehr et al. 2001).

5.3.4 Management

Elimination diet

The mainstay of the management of food allergy is avoidance of the causal food (Sampson and McCaskill 1985, Pastorello et al. 1989, Chehade 2007). This involves extensive education of patients and their parents about the proper reading of packaged food labels. In placebo-controlled studies on the effects of elimination diets the AD of children improved significantly after eliminating egg, milk and/or peanut (Atherton et al. 1978, Sampson and McCaskill 1985, Lever et al. 1998). On the other hand, there is one double-blind placebo-controlled study with egg and milk showing that the majority of the children with AD did not benefit from the elimination diet (Neild et al 1986).

When multiple foods are eliminated from the diet, it is prudent to make a tailor-made, nutritionally-balanced diet with the aid of an experienced physician and dietitian. The patients and their parents need support, advice and education about food allergies. The first aid must be advice on how to treat severe reactions

caused by accidental ingestion of an eliminated food item such as cow's milk or peanut (Sampson 2004, Sicherer and Sampson 2006).

Specific oral tolerance induction and Anti-IgE therapy

Recently, specific oral tolerance induction has been tried as an experimental management for food allergy, mainly in older children with IgE-mediated allergy to cow's milk and egg. Desensitization is achieved by eating increasing doses of the specific food allergen regularly over months in the induction phase. Once the maximum dose has been achieved, the regular maintenance dose is consumed daily. Preliminary studies on milk and egg resulted in increased tolerance in 64-86% of children at the time of re-challenge (Meglio et al. 2004, Buchanan et al. 2007, Staden et al. 2007). The increased threshold obtained for a given food reduces the risk of severe allergic reactions. However, adverse events during the desensitization treatment are frequent, which may affect compliance and also means supervision by an experienced allergologist is required (Staden et al. 2007).

In one study, patients with severe peanut allergy received monthly injections of anti-IgE monoclonal antibodies (TNX-901) which increased the threshold to ingested peanut in a majority of the patients (Leung et al. 2003). Omalizumab has also been tried in peanut allergy, but the phase II trial had to be discontinued because of concerns over the safety of the oral peanut challenges in some patients and thus it is not indicated for the treatment of food allergy so far (Sampson 2007). Omalizumab treatment can rarely be associated with anaphylaxis (Cox et al. 2007). It has to be administered at regular intervals to maintain its protective effect and it is rather expensive, due to which its use is limited.

5.3.5 Immunopathogenesis

The gastrointestinal tract is considered as the largest immunologic organ in the body. It is constantly bombarded by an innumerable amount of dietary proteins. Ingested dietary proteins are subjected to degradation and destruction by gastric acidity and luminal digestive enzymes, resulting normally in the destruction of

immunogenic epitopes. In animal models a disturbance in these factors (gastric acidity, digestive enzymes) has been shown to lead to food sensitization rather than tolerance. Other factors affecting proteins in the lumen are gastrointestinal peristalsis and the protective mucus layer that lines the intestinal epithelium (Chehade and Mayer 2005).

Antigens can be taken up in the gut at least at three different sites, by microfold cell (M cell), dendritic cell (DC) and epithelial cell routes. Peyer's patches (PPs) are overlaid by specialized epithelial cells, called M cells. Antigens are taken up by M cells overlying PPs and then delivered to DCs in the subepithelial region. That, in turn, ingests the antigen and delivers it to the underlying B-cell follicles of the PPs where the production of IgA occurs. The second site of antigen sampling is DCs which are present in different compartments of the gut, including the intestinal lamina propria, PPs and mesenteric lymph nodes. These potent antigen-presenting cells (APCs) can send dendrites into the lumen and sample antigen directly from the lumen. Antigen-carrying DCs may then go through the lymphatics to the mesenteric lymph nodes, but this has not been definitely shown. Soluble antigens may cross the intestinal epithelial cells through transcellular routes, encountering T cells or macrophages in the lamina propria, or a paracellular route reaching the circulation (Chehade and Mayer 2005).

Oral tolerance can be induced in mice after administration of either a single high dose or repeated lower doses of food antigen (Friedman and Weiner 1994). A high dose of oral antigen leads to lymphocyte anergy or deletion. A low dose results in tolerance by activation of regulatory T cells. Other factors that are involved in the induction of oral tolerance are the genetics of the host, the gut microbiota of the host, the form of the antigen and the age of the host. Infants have stronger immunologic reactions to dietary antigens during the first three months of life. It can be concluded that a failure of induction of oral tolerance or breakdown in oral tolerance mechanisms results in food allergy (Chehade and Mayer 2005).

Food allergy may also result from sensitization through a respiratory route. For instance, pollens are able to induce the formation of IgE antibodies that recognize homologous epitopes on food proteins of plant origin and cause a pollen-food allergy syndrome (Breiteneder and Ebner 2000). Birch pollen-specific T cell responses have been found from the skin lesions of patients with AD and sensitized to birch pollen. These patients reacted with worsening of AD after oral challenge with birch pollen-related foods (Reekers et al. 1999). Werfel et al. found significant differences in the proliferative response of blood lymphocytes between patients who reacted to milk with worsening of AD and controls. They were also able to generate casein-specific T cell clones from the blood of these patients. These findings stress that a non-IgE mediated mechanism may be involved in the eczematous reaction to food, indicating the role of allergen-specific T lymphocytes in AD (Reekers et al. 1996, Werfel et al. 1996, Werfel et al. 1997).

5.4 Food allergens

In type I allergy, the allergen has the property of inducing the immune system to produce IgE antibodies (i.e., to sensitize) and triggering allergic symptoms in a sensitized individual (Aalberse 2000). Typical common food allergens are identified as water-soluble glycoproteins 10 to 70 kilodaltons (kDa) in size that are stable to heat, acid and proteases (Kay 2001, Sicherer and Sampson 2006). There is however no single structural, functional or chemical property that determines that a protein is an allergen (Aalberse 2000, Pomés 2002). Allergens that are recognized by more than 50% of sensitized individuals are considered as major allergens, and those recognized by less than 50% of these individuals are termed minor allergens (Liebers et al. 1996). The Allergen Nomenclature Sub-Committee of the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS) maintain an official database of all identified allergens at <http://www.allergen.org>. Allergens are designated according to the taxonomic name, so that the first three letters of the genus are followed by the first letter of the species name and an Arabic number indicating the chronology of allergen purification (Chapman et al. 2007)

5.4.1 Plant food allergens

The plant-derived food allergens are classified into families and superfamilies depending on their structural and functional properties (Breiteneder and Radauer 2004). The largest groups of plant proteins that contain allergens are the cupin and prolamin superfamilies and the protein families of the pathogenesis-related proteins. The cupin superfamily includes vicilins and legumins. Examples of these families are respectively the major peanut allergen Ara h 1, which is a 7S globulin, and the major hazelnut allergen Cor a 9, which belong to the 11S globulins. The prolamin superfamily includes allergens such as the nonspecific lipid transfer proteins (for example, in apple, Mal d 3), the cereal α -amylases (barley, Hor v 15), protease inhibitors, prolamins (wheat, Tri a 19) and 2S albumins. The latter form a major group of storage proteins and typical 2S albumins are heterodimeric proteins consisting of two polypeptide chains of approximately 4 and 9 kD held together by four disulfide bonds (Shewry et al. 1995). Several peanut allergens (Ara h 2, 6, 7), tree nut allergens (walnut, Jug r 1) and seed allergens (sesame, Ses i 1, 2) are 2S albumins (Breiteneder and Radauer 2004, Radauer and Breiteneder 2007).

Pathogenesis-related proteins represent a collection of 14 unrelated protein families that function as part of the plant defence system. The families which are included in this group are, for example, class I chitinases (avocado, Pers a 1), thaumatin-like proteins (apple, Mal d 2), proteases (kiwi, Act c 1) and Kunitz-type protease inhibitors (potato, Sola t 2) (Seppälä et al. 1999, Breiteneder and Radauer 2004, Radauer and Breiteneder 2007).

5.4.2 Animal food allergens

The most important animal food allergens are present in egg, milk and seafood. These allergens include muscle proteins, enzymes and serum proteins (Sicherer 2001, Chapman et al. 2007). The parvalbumins are major allergens in fish (codfish, salmon) and cross-reactivity among different fish species may exist (Van Do et al. 2005). Invertebrate tropomyosin is a panallergen in *Crustacea* showing sequence homology in shrimp, crab, lobster and molluscs (Leung et al. 1996).

5.4.3 Food allergen cross-reactivity

The phenomenon of allergen cross-reactivity occurs when IgE antibodies primary raised against one allergen recognize a protein from another source that has a high degree of homology (Aalberse et al. 2001, Ferreira 2004). The major allergen of birch pollen, Bet v 1, belongs to the pathogenesis-related proteins (PR-10) and is the most frequent cause of the pollen-food-related allergy syndrome. The typical cross-reactive foods are carrot (Dau c 1) and apple (Mal d 1). These proteins are sensitive to heat denaturation and thus clinical manifestations are elicited mainly by raw fruits and vegetables (Breiteneder and Radauer 2004). Profilins are ubiquitous cross-reactive plant allergens which are found in birch pollen (Bet v 2) with homologous proteins in apple, carrot and celery. Profilins are also involved in the mugwort-celery-spice syndrome and cross-reaction between grass pollen and food (Breiteneder and Ebner 2000).

Table 1. Characterized food allergens

Food	Allergen	Family	
Basic foods			
Cow's milk	Bos d 4	α -lactalbumin	
	Bos d 5	β -lactoglobulin	
	Bos d 8	Casein	
Hen's egg	Gal d 1	Ovomucoid	
	Gal d 2	Ovalbumin	
	Gal d 3	Ovotransferrin	
	Gal d 4	Lysozyme	
	Gal d 5	α -livetin	
Wheat	Tri a 14	LTP	
	Tri a 19(ω -5 gliadin)	Prolamin	
	Tri a 26	Glutenin	
Rye	Sec c 20	Prolamin	
Barley	Hor v 15	α -amylase inhibitor	
	Hor v 16	β -amylase inhibitor	
	Hor v 17	Prolamin	
Soybean	Gly m 3	Profilin	*
	Gly m 4	PR-10	*

Fish	Gad c 1 Sal s 1	Parvalbumin Parvalbumin	
Shrimp	Pen a 1	Tropomyosin	
Vegetables and fruits			
Potato	Sola t 1 Sola t 2 Sola t 3 Sola t 4	Patatin Kunitz-type	
Carrot	Dau c 1 Dau c 4	Protease inhibitor PR-10 Profilin	* *
Celery	Api g 1 Api g 4	PR-10 Profilin	* *
Apple	Mal d 1 Mal d 2 Mal d 3 Mal d 4	PR-10 PR-5 LTP Profilin	* *
Pear	Pyr c 4	Profilin	*
Cherry	Pru av 1 Pru av 2 Pru av 3 Pru av 4	PR-10 PR-5 LTP Profilin	* *
Peach	Pru p 1 Pru p 3 Pru p 4	PR-10 LTP Profilin	* *
Banana	Mus xp 1	Profilin	*
Avocado	Pers a 1	PR-3	
Nuts			
Peanut	Ara h 1 Ara h 2 Ara h 3 Ara h 4 Ara h 5 Ara h 6 Ara h 7 Ara h 8 Ara h 9	Vicilin 2 S albumin Legumin Legumin Profilin 2 S albumin 2 S albumin PR-10 LTP	 * *
Hazelnut	Cor a 1 Cor a 2 Cor a 8 Cor a 9 Cor a 11	PR-10 Profilin LTP Legumin Vicilin	* *
Cashew nut	Ana o 1 Ana o 2 Ana o 3	Vicilin Legumin 2 S albumin	

Walnut	Jug r 1	2 S albumin
	Jug r 2	Vicilin
	Jug r 3	LTP
Chestnut	Cas s 5	PR-3
	Cas s 8	LTP
Brazil nut	Ber e 1	2 S albumin
	Ber e 2	Legumin

PR =Pathogenesis-related proteins, LTP=Nonspecific lipid transfer proteins

*Cross-reaction to birch and/or mugwort pollen

5.5 Allergy to mustard and other seeds

There are three different mustard seeds: yellow (*Sinapis alba*), oriental (*Brassica juncea*) and black (*Brassica nigra*). Yellow and oriental mustard seeds are used in foods as such, ground into powder or processed into prepared table mustard. Black mustard is used only in the pharmaceutical industry. Over the past 25 years several cases of anaphylaxis to mustard have been reported (Panconesi et al. 1980, Widström et al. 1986, Monreal et al. 1992, Jorro et al. 1995, Kanny et al. 1995, Caballero et al. 2002). The prevalence of mustard allergy in French children suspected of food allergy was 6-8.9% confirmed by food challenge. It has been found to be the most prevalent food allergen in French children after egg, peanut and milk (Rancé et al. 1999a, Rancé et al. 1999b, Rancé and Dutau 2002). Two French studies showed that 23% - 42% of children and adolescents with positive SPT to mustard had a positive immediate oral food challenge reaction to mustard (Rancé et al. 2000, Morisset et al. 2003a). It is one of the most common spice allergens in adults (Niinimäki et al. 1989, Niinimäki et al. 1995). Mustard contains irritants such as capsaicin and isothiocyanates which may be responsible for false positive SPT and labial challenge results (Rancé 2003). In the study by Rancé et al. mustard allergy started before the age of 3 years in the majority of the children investigated (Rancé et al. 2000). Recently it has been included in the list of potential allergenic foods that must be notified in food labeling in the European Union. A Spanish study with mainly adult patients allergic to mustard suggested that there might be an association between mustard hypersensitivity and mugwort pollen (Figueroa et al. 2005). In the Finnish

studies sensitization to mustard in adult patients was associated with birch pollen sensitization, but not so closely as the other spices (coriander and caraway) (Niinimäki and Hannuksela 1981, Niinimäki et al. 1995). Contact allergy to mustard and respiratory allergy to *Sinapis alba* pollen have been reported, but are very rare (Dannaker and White 1987, Kavli and Moseng 1987, Anguita et al. 2007).

Foods that may contain sesame seeds are bakery products, vegetarian foods, muesli, dips, salad dressings and Middle Eastern dishes (halva, tahini). In 1950, Rubenstein reported a case of an adult patient who experienced anaphylaxis after eating halva and other food containing sesame (Rubenstein 1950). After that, other cases of anaphylaxis caused by sesame have been reported in children and adults (Kägi and Wüthrich 1993, Dalal et al. 2003, Agne et al. 2004, Derby et al. 2005). The frequency of allergy to sesame seed in children suspected of food allergy in France was 0.6% in a population of 544 children (Rancé et al. 1999a). In Israel the prevalence of sesame seed sensitization in children suspected of food allergy was 1.2%, next to egg and milk. In that study, six children had suffered sesame-induced anaphylaxis (Dalal et al. 2002). In another Israeli study the sesame allergy started in the majority of the children before two years of age and persisted in 80% of the allergic children when followed for an average of 6.7 years (Cohen et al. 2007). Allergy to sunflower seed is relatively rare, but food allergy, anaphylaxis and contact urticaria have been observed in a few cases (Noyes et al. 1979, Halsey et al. 1986, Axelsson and Zetterström 1994, Durans et al. 1997, Palma-Carlos et al. 2005).

Table 2. Characterized seed allergens

Food	Allergen	Family
Seeds		
Sesame	Ses i 1	2 S albumin
	Ses i 2	2 S albumin
	Ses i 3	Vicilin
	Ses i 4	Oleosin
	Ses i 5	Oleosin
	Ses i 6	Legumin
	Ses i 7	Legumin
Mustard	Sin a 1	2 S albumin
	Sin a 2	Legumin
	Bra j 1	2 S albumin
Sunflower	Hel a 3	LTP

5.6 Turnip rape and oilseed rape allergy

Oilseed rape (*Brassica napus* ssp. *oleifera*) is derived from swede and turnip rape (*Brassica rapa* ssp. *oleifera*, formerly *Brassica campestris*) from turnip (Table 3).

Table 3. Plants in the *Brassicaceae* family.

Plant	Species
Swede	<i>Brassica napus</i>
Oilseed rape	<i>Brassica napus</i> ssp. <i>oleifera</i>
Turnip	<i>Brassica rapa</i>
Turnip rape	<i>Brassica rapa</i> ssp. <i>oleifera</i>
Cabbage	<i>Brassica oleracea</i>
Oriental mustard	<i>Brassica juncea</i>
Black mustard	<i>Brassica nigra</i>

Both plants are bright yellow flowering members of the *Brassicaceae* family (Fig. 2) widely cultivated throughout the world for the production of animal feed, biodiesel and vegetable oil for human consumption. Rapeseed oil, which is usually a mixture of turnip rape and oilseed rape, is the third leading source of vegetable oil in the world after soy and palm oil. Rapeseed oil is widely used in

human consumption for its health effects and nutritional value (Dupont et al. 1989). A few case reports of occupational allergies to oilseed rape dust have been published (Monsalve et al. 1997, Suh et al. 1998, Alvarez et al. 2001). In one of these case reports, a hydrophilic 2S albumin was shown to be allergenic for one patient (Monsalve et al. 1997). In addition, allergy to oilseed rape pollen has been widely discussed. It seems to be rare, because very little airborne pollen is transported over longer distances (Hemmer 1998). The prevalence was less than 0.2% in an English study unless the patients were occupationally exposed (Fell et al. 1992). The allergens identified in oilseed rape pollen contain profilins, calcium-binding proteins and pectinase (Focke et al. 1998, Chardin et al. 2003).

Besides 2S albumins from mustard (Table 2), allergens in turnip have been identified as prohevein (Bra r 2) and in cabbage as LTP (Bra o 3) (Hänninen et al. 1999, Palacin et al. 2006).

Figure 2. Flowers of oilseed rape



6. AIMS OF THE PRESENT STUDY

The aims of the present study were:

1. To study the prevalence of sensitization to turnip rape and oilseed rape in children with atopic dermatitis and to examine by food challenge whether the sensitized children get symptoms from turnip rape seeds.
2. To investigate whether the sensitization to turnip rape and oilseed rape are associated with other food allergies or atopic disorders.
3. To compare turnip rape, oilseed rape and mustard sensitization and food challenges in Finnish and French children with AD and to investigate allergen cross-reactivity by laboratory experiments.
4. To characterize the major allergens in turnip rape and oilseed rape.

7. PATIENTS AND METHODS

7.1 Patients and control children

7.1.1 Patients (I, II, III, IV)

The patients and control children included in the four studies (I-IV) of the present thesis are shown in Table 4.

Table 4. Study designs (I-IV), sensitized children and control children.

Study	Children N (girl/boy)	Age, mean (range), years
<i>I Prevalence of sensitization to turnip rape/oilseed rape</i>		
Total number of children with suspected food allergy	1887 (781/1106)	< 16
Food challenge to turnip rape	28 (12/16)	4.8 (1.0- 15.4)
Control children	25 (10/15)	3.2 (0.5- 9.4)
<i>II Associated food allergies and atopic diseases in children sensitized to turnip rape and oilseed rape</i>		
Sensitized children	64 (21/43)	2.5 (0.5- 5.5)
Age-matched control children	64 (21/43)	
<i>III Relationship between mustard and turnip rape allergy</i>		
Sensitized children	Finnish 14 (5/9)	3.8 (1.3- 9.1)
	French 14 (4/10)	4.7 (1.5- 9.3)
Age-matched control children	28 (9/19)	
<i>IV Detection of major allergens in turnip rape- and oilseed rape- sensitized children</i>		

Sensitized children	72 (25/47)	3.3 (0.5- 15.4)
Age-matched control children	72 (25/47)	

During a two-year period from 2002 to 2004, a total of 1887 Finnish children under 16 years of age were evaluated with turnip rape and oilseed rape SPT (I). They all had been referred to the Department of Dermatology at Tampere University Hospital for suspected food allergy. Most of these children had AD and 70% (1337) of them were below three years of age. From this population, 28 children with AD and SPT to turnip rape and/or oilseed rape with a mean wheal diameter ≥ 5 mm were enrolled in the turnip rape challenge study. Seventeen of them were allergic to cow's milk and 24 to wheat, as confirmed by open food challenges.

Sixty-four Finnish children with AD and a positive SPT (mean wheal diameter ≥ 5 mm) to turnip rape and/or oilseed rape were included in the case-control study (II). Forty-six (72%) of them were allergic to cow's milk and fifty-one (80%) to wheat. A structured questionnaire was used to collect data on breast-feeding, associated allergic rhinitis or asthma, sensitization to other foods or pollens and on family history of atopic disorders.

Fourteen Finnish children with AD were examined at Tampere University Hospital and 14 French children at the Department of Respiratory and Allergic Diseases in Children and Adolescents, University Hospital Purpan, Toulouse, France (III). Inclusion criteria for the turnip rape and mustard challenge study were AD and a positive SPT (mean wheal diameter ≥ 3 mm) to turnip rape. All 14 Finnish children were allergic to wheat and 12 to cow's milk. Thirteen of the French children were allergic to egg, eight to peanut and three to cow's milk.

In study IV, sera were obtained from 72 Finnish children with AD and a positive SPT (mean wheal diameter ≥ 5 mm) to turnip rape and/or oilseed rape. Fifty-two (72%) were allergic to cow's milk and wheat. These children were chosen from the total of 1887 children evaluated.

7.1.2 Control children (I-IV)

All control patients were collected from the Department of Dermatology at Tampere University Hospital where they had been referred for food allergy. All of them had AD and negative SPTs (mean wheal diameter ≤ 2 mm) to turnip rape and oilseed rape. False positive irritant reactions in the labial turnip rape challenge were excluded by showing negative challenge reactions in 25 atopic control children (I). Sixty percent of them had allergy to cow's milk and 48% to wheat, as confirmed by open food challenges. In addition, 60% were sensitized to egg. Sixty-four age- and sex-matched children were included as controls in the case-control study (II). These children were examined in the same period as the cases and were selected randomly from the files by matching the sex and age (± 6 months). Twenty-three (36%) of them had allergy to cow's milk and twenty-one (33%) to wheat. In study III, 28 age- and sex-matched children served as controls in the IgE antibody tests. Control sera were collected in study IV from 72 age- and sex matched children. Twenty-five (35%) of them were allergic to cow's milk, 24 (33%) to wheat and 28 (39%) were sensitized to egg.

7.1.3 Ethics

The study protocols were approved by the Ethics Committees of Tampere University Hospital and the University Hospital of Purpan, Toulouse. Informed written consent was obtained from the parents.

7.2 Methods

7.2.1 Challenge tests (I, III)

The turnip rape and mustard food challenges were open. The turnip rape challenge started with the application of a small amount of oily mass of crushed turnip rape seeds on the lip (Rancé and Dutau 1997). The test was considered positive when wheals showed up on the lips or surrounding cutaneous area, or when clear labial swelling was seen. The challenge was followed, if negative, by giving increasing doses of crushed turnip rape seeds mixed in appropriate food (10, 10, 30, 100, 300 and 900 mg) orally every 20 min. If negative on the first day, the challenge was continued at home for six days. The daily dose was 1350

mg of turnip rape. The severity of AD was scored at the beginning and at the end of the challenge by SCORAD (Severity scoring of atopic dermatitis 1993). In study III, the mustard challenge was performed at least a week after the turnip rape challenge. A labial challenge was not done due to the irritant effect of mustard (Rancé 2003). Increasing doses of crushed mustard (*Sinapis alba*) seeds mixed in an appropriate food were given orally every 20 min. The oral doses were 1, 5, 10, 20, 50, 100, 250 and 500 mg. All cutaneous, respiratory and gastrointestinal symptoms were registered during the challenges. When a positive reaction appeared, the challenge was stopped and the child received proper medication.

7.2.2 Skin prick tests (I-IV)

Seeds of turnip rape (*Brassica rapa* ssp. *oleifera* cv Kulta) and oilseed rape (*B. napus* ssp. *oleifera* cv Hyola 38) obtained from Mildola Oy (Kirkkonummi, Finland) were ground. SPT was performed with crushed seeds of turnip rape, oilseed rape and mustard (*S. alba*) moistened with physiological saline using a commercial one-peak lancet (ALK-Abelló A/S, Hørsholm, Denmark) and the prick-prick method (Dreborg and Foucard 1983). Purified proteins from turnip rape and oilseed rape diluted in PBS (10 mM sodium phosphate and 0.15 M NaCl, pH 7.4) were tested in concentrations ranging from 10 to 100 µg/mL. Histamine dihydrochloride (10mg/mL, ALK-Abelló) was used as a positive and saline (Soluprick SQ, ALK-Abelló) as a negative control.

7.2.3 IgE antibodies to oilseed rape and mustard (I-IV)

Sera collected from all sensitized and control children were stored at -20°C until testing. IgE antibodies to oilseed rape (f316) and mustard (f89) were measured using ImmunoCAP (Specific IgE, Phadia Ab, Uppsala, Sweden). Values ≥ 0.35 kU/l were considered positive according to the manufacturer's instructions. No commercial allergen to turnip rape was available.

7.2.4 ELISA (III, IV) and ELISA inhibition (III)

IgE antibodies to 2S albumins of turnip rape (Bra r 1), oilseed rape (Bra n 1) and mustard (Sin a 1) were examined by ELISA. Microtitre plates were coated with 2S albumins purified from the seeds of these plants by gel filtration and cation

exchange chromatography. Microtiter plates were coated with the allergen solutions 1 µg/mL, 100 µl/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 (para-nitro-phenyl phosphate, 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 absorbance of the 28 control children in study III were considered positive. In study IV, the mean value of the control sera plus 3 SDs was used as the cut-off limit for a positive result.

Inhibition experiments by ELISA were performed to determine IgE cross reactivity between the three allergens. Coating and post-coating were performed as described above, but the volume of allergen added was 50 µL 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 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.

7.2.5 Characterization of allergens (IV)

The ground seeds of turnip rape and oilseed rape were extracted overnight with PBS at room temperature. The samples were centrifuged and the supernatant was collected and filtered through a 0.4 µm filter (Millex HV, Millipore, Bedford, MA, USA). For protein purification, the clarified seed extracts were subjected to gel filtration, cation exchange chromatography and reversed phase chromatography. Chromatography fractions from turnip rape and oilseed rape were analyzed by electrophoresis and immunoblotting. Proteins separated by SDS-PAGE were transferred to a polyvinylidene difluoride (PVDF) membrane

(Immobilon Transfer Membranes, Millipore, Bedford, MA, USA) and treated with a pool of sera from 8 children with positive specific IgE ELISA (turnip rape and oilseed rape) results. After incubation, biotinylated anti-human IgE diluted 1:1000 (Vector laboratories, Inc, Burlingame, CA, USA) was added, followed by streptavidin-conjugated alkaline phosphatase and substrate. Mass spectrometry was performed with an Ultraflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)/TOF mass spectrometer (Bruker-Daltonics, Bremen, Germany) equipped with a nitrogen laser. N-terminal sequencing was performed using Edman degradation.

7.2.6 Statistical analyses (I-IV)

In study I, the statistical comparison was made using the permutation test. Correlation coefficients were calculated by the Spearman method.

In study II, univariate and stepwise (forward selection) conditional logistic models with exact inference (Monte Carlo) were used to analyse the cases matched to the controls. The statistical comparison between cases and controls was made using the permutation test with the Monte Carlo p-value. The results were expressed as medians with interquartile ranges (IQR).

In study III, the statistical comparison was made using Fisher's exact test and the permutation test. The results were expressed as medians with interquartile ranges (IQR).

In study IV, the statistical comparison was made using the Mann-Whitney U and Kruskal-Wallis tests. The post-hoc testing of several univariate comparisons was performed with the Conover-Inman procedure.

8. RESULTS

8.1 Turnip rape and oilseed rape sensitization and allergy in children with AD (I)

Sensitization in skin prick-tested children

Two hundred and six (10.9%) of the 1887 children screened during a two-year period had a positive ($\geq 5\text{mm}$) SPT to turnip rape (9.3%) or oilseed rape (9.4%). One hundred and forty-nine (11%) of the children under three years of age were sensitized to turnip rape and/or oilseed rape.

Challenge reactions in sensitized children

Twenty-five (89%) of the 28 challenged children had a positive challenge reaction to turnip rape. The labial challenge was positive in 17 (68%) children, showing whealing and/or swelling. One of them also got rhinitis. The oral challenge was positive in eight children. Four of the children got immediate symptoms in < 3 hours and another four during the challenges at home. Two of the children with immediate oral reactions got facial urticaria and the other two showed generalised erythema and abdominal complaints. The symptoms in the children with delayed positive oral challenge reactions were flareup of AD and one child got exacerbation of asthma and abdominal symptoms (I; Table 2). All 25 control children remained negative in the turnip rape labial challenge.



Figure 3.
SPT reactions of patient 1 to

1. Histamine 5 mm,
2. Neg. control 0.
3. Crushed turnip rape seeds 10 mm
4. Crushed oilseed rape seeds 25 mm
5. Mustard 15 mm
6. Crushed turnip rape seeds (crushed finer) 23 mm
7. Crude turnip rape oil 0.

Figure 4. Positive labial challenge reaction (swollen upper lip and local urticaria) to crushed turnip rape seeds in patient 1.



SPT reactions and specific IgE in challenged children

There was a clear correlation between the size of turnip rape and oilseed rape SPTs [0.69 (95% CI: 0.43 to 0.95)] in the 28 children challenged with turnip rape. The SPT reactivity to turnip rape and IgE antibody levels to oilseed rape in the challenged children is shown in Fig. 2 (I). At the time of challenge the SPT reaction to turnip rape and oilseed rape had decreased to less than 5 mm in two children, whereas in all the other children the SPT was still clearly positive. The first child with a small SPT reaction showed flareup of AD, whereas in the second child the turnip rape challenge was negative. Only one of the 25 control children had IgE antibodies (2.1 kU/l) to oilseed rape. The total IgE was 1510 kU/l (median; 95% CI: 634 to 1790 kU/l) in the 25 challenge-positive children. In the three challenge-negative children, the total IgE was 26 kU/l, 27 kU/l and 323 kU/l, respectively.

8.2 Case-control study in children with AD sensitized to turnip rape and oilseed rape (II)

The univariate analysis showed statistically significant differences between the 64 children with AD sensitized to oilseed rape and turnip rape and the non-sensitized control children in all parameters except family history of atopy (II; Table 1). In the cases the median exclusive breast-feeding time was four months (IQR 3.0 to 4.4 months) which was significantly longer ($p=0.044$) than the median of the controls which was three months (IQR 2 to 4 months). AD appeared at a mean age of 3 months (range 2 weeks - 9 months) in the cases and 7.5 months (range 2 weeks - 36 months) in the controls. Associated asthma ($p<0.001$) and allergic rhinitis ($p<0.01$) were more common in the sensitized children than in the controls.

Almost all the children sensitized to oilseed rape and turnip rape were sensitized to mustard (97%) and egg (92%), which gave odds ratios of 61 and 36, respectively. In agreement with this, the forward stepwise model showed that

only these two foods entered into the model. The cases were more often sensitized to pollens, especially to birch (OR 11.5), than the controls.

8.3 Turnip rape and mustard allergy in Finnish and French children with AD (III)

Challenge reactions to turnip rape and mustard

All 14 (100%) Finnish children and five (36%) French children had positive food challenge to turnip rape (III; Table 2). Twelve Finnish children and five French children showed positive labial challenge. In addition, two Finnish children reacted in oral challenge to 40 mg and 140 mg of turnip rape. The oral mustard challenge was positive in five (36%) Finnish and five (36%) French children. These five French children had a 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. The symptoms included urticaria in three Finnish and two French children. Two Finnish and two French children showed abdominal complaints and one French child had an asthmatic reaction requiring a bronchodilator.

Skin prick test results

Because of 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 (III; Table 2). The SPT to oilseed rape was positive in 12 (86%) Finnish (mean wheal diameter 4.4 mm) and 10 (71%) French (mean wheal diameter 6.1 mm) children. The SPT to mustard was positive in all Finnish (mean wheal diameter 5.7 mm) and French (mean wheal diameter 7.1 mm) children. All 28 control children had negative SPTs to turnip rape, oilseed rape and mustard.

IgE and ELISA measurements

IgE ImmunoCAP to oilseed rape was positive in 14 (100%) Finnish and 10 (71%) French children. In the Finnish children the median IgE level to oilseed rape was 4.6 kU/l (IQR: 1.7, 29 kU/l) 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 (III; Table 2). The median IgE levels to mustard were 3 kU/l (IQR: 1, 18.8 kU/l) and 3.6 kU/l (IQR: <0.35, 10 kU/l), respectively. 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.

2S albumin IgE ELISA was positive to turnip rape in 10 (71%) Finnish and eight (57%) French children, to oilseed rape in 10 (71%) Finnish and eight (57%) French children, and to mustard in nine (64%) Finnish and seven (50%) French children. The median IgE level to 2S albumins of turnip rape in the Finnish children 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. In both Finnish and French children all three 2S albumin IgE levels were significantly ($P<0.001$) higher than in the control children.

ELISA inhibition measurements

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 patients' sera to solid-phase allergens was effectively inhibited in a dose-dependent manner by all soluble inhibitors. Inhibition curves in (III; 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 very 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.

8.4 Characterization of oilseed rape and turnip rape allergens (IV)

When seed extracts from oilseed rape and turnip rape were subjected to SDS-PAGE and IgE immunoblotting with patient sera, the major reactivity was in the

protein area between 6 and 14 kDa. To purify the major immunoreactive proteins, the seed extracts were subjected to gel filtration and then the immunoreactive fractions were subjected to cation exchange chromatography (IV; Fig. 1). Four fractions from oilseed rape and 2 fractions from turnip rape were selected for further analyses. SDS-PAGE and immunoblot analyses of these fractions resulted in very similar patterns, with 2 major protein bands of sizes approximately 4 and 9 kDa. For identification, the proteins from cation exchange chromatography fractions were, after SDS-PAGE, electroblotted onto a PVDF membrane and subjected to N-terminal sequence analysis. For comparison, 2S albumin from yellow mustard (*Sinapis alba*) was purified using the same methods. In general, all the determined N-terminal sequences of the 2S albumin large subunits from turnip rape, oilseed rape and mustard were identical. MALDI-TOF mass spectrometric analysis of the turnip rape and oilseed rape 2S albumins from different cation exchange fractions showed molecular masses between 9.5 to 14.5 kDa. This is in accordance with the expected molecular masses of 2S albumins consisting of one small and one large subunit. IgE ELISA assays were performed with fractions from cation exchange chromatography and approximately 80% of the 72 children had IgE to purified 2 S albumins from turnip rape and oilseed rape. SPTs to two purified fractions of turnip rape and four purified fractions of oilseed rape showed positive reactions in all six children tested (IV; Table II).

9. DISCUSSION

In developed countries the prevalence of AD in children is almost 20% (Leung and Bieber 2003, Asher et al. 2006) and the prevalence of food allergy 2 to 8% (Bock 1987, Roehr et al. 2004, Zuberbier et al. 2004, Pereira et al. 2005). In children with moderate to severe AD, food allergy is common (Akdis et al. 2006, Hill et al. 2008). Relatively few foods encompass most food allergies despite the enormous diversity of the diet. Cow's milk, egg, fish, soy, wheat and peanut account for about 90% of reported food allergies in infants and young children (Burks et al. 1998, Rancé 1999a). Sesame seed and mustard allergies are examples of new, important food allergens in children. Turnip rape and oilseed rape have not been considered as potent food allergens before and, although allergy to oilseed rape pollen has been discussed, it seems to be rare (Fell et al. 1992).

9.1 Turnip rape as a new food allergen: sensitization patterns, challenge results and patient profiles (I, II)

In the first study we found a prevalence as high as 11% of positive SPT (≥ 5 mm) reactions to turnip rape or oilseed rape in a cohort of 1887 Finnish children examined for suspected food allergy. Twenty-eight children with AD and positive SPTs to turnip rape and oilseed rape participated in the open turnip rape challenge study. Twenty-five children with AD and negative SPT to turnip rape and oilseed rape served as controls in the labial challenge to turnip rape. Most (89%) of the sensitized children showed positive labial or oral challenge reactions to turnip rape in contrast to none of the control children. The challenge reactions were mostly of an immediate type such as labial swelling or facial urticaria. The dose of crushed turnip-rape seeds causing a positive oral challenge

varied from 20 to 1350 mg. The variation in the cumulative reactive doses correlates well with previous results in peanut and sesame seed challenge studies (Morisset et al. 2003b). Three challenge-positive children showed flareup of AD when the oral challenge had lasted for 2-5 days. Similar delayed-type challenge reactions have been observed previously with cow's milk and pollen-related foods (Isolauri and Turjanmaa 1996, Breuer et al. 2004b). When the 25 challenge-positive and three challenge-negative children were compared, the latter had smaller SPT reactions to turnip rape and oilseed rape and also lower IgE antibody levels to oilseed rape, suggesting a lower sensitization level to these plants. There was a tendency that younger children showed larger SPT reactions and immediate-type responses to smaller amounts of turnip rape seeds than older children, but the numbers of children in the variously reacting challenge groups were small. The high correlation in the SPT reactivity between oilseed rape and turnip rape shown in study I suggests allergic cross-reactivity, which is supported by the finding in study IV of highly homologous 2S albumin allergens in both of them.

The seeds of these oilseed plants are not eaten in the same way as sesame or other seeds causing food allergy (Dalal et al. 2002, Derby et al. 2005). Therefore we studied other possibilities that could lead to sensitization to them. The children turned out to be sensitized to all the pollens and foods tested significantly more frequently than the control children. The high sensitization rate (97%) to mustard in the studied children was not unexpected, because the seeds of mustard contain 2S albumins highly homologous with oilseed rape and turnip rape as shown in studies III and IV. Mustard is a common food allergen in children and adults in France (Rancé et al. 2000, Morisset et al. 2003a). Its consumption is, however, minimal in young children or their lactating mothers in Finland and therefore it is highly unlikely that mustard is the primary cause of the frequent sensitization to oilseed rape and turnip rape in the present children. A significant (97%) association between mustard hypersensitivity and sensitization to mugwort pollen in adults has been reported from Spain (Figuerola et al. 2005). In contrast, only 28% of the present children were sensitized to mugwort and there is no evidence to our knowledge that mugwort pollen

contains 2S albumins. In two Finnish studies, sensitization to mustard in adult patients was associated with birch pollen sensitization, but not so closely as the other spices (Niinimäki and Hannuksela 1981, Niinimäki et al. 1995). Like birch pollen, oilseed rape pollen contains profilin which may rarely cause airway symptoms (Focke et al. 1998, Murphy 1999).

In addition to mustard, oilseed rape and turnip rape sensitization was strongly (OR 36) associated with a positive SPT reaction and IgE antibodies to egg. This finding cannot be explained by any molecular cross-reactivity, but it is noteworthy that IgE antibodies to egg in infancy predict later development of atopic disease (Nickel et al. 1997, Peroni et al. 2007). Egg sensitization in children is also a typical sign of a tendency toward other IgE sensitizations (Nickel et al. 1997). In agreement with this, the egg- and oilseed plant-sensitized children had associated asthma and allergic rhinitis significantly more often than the control children.

Prolonged exclusive breast-feeding may increase sensitization to cow's milk (Friedman and Zeiger 2005) and may have an effect on the appearance of AD (Bergmann et al. 2002, Siltanen et al. 2003). Though allergy to cow's milk showed only a weak association (OR 3.6) to oilseed plant sensitization, the present children had had exclusive breast-feeding for a significantly longer time than the control children. The median breast-feeding times were 4 and 3 months but, because the data was collected retrospectively, it may contain some sampling bias. However, we cannot exclude the possibility that the present children were sensitized to oilseed plant allergens in the mother's milk. Supporting this, a few of the sensitized infants were exclusively breast-fed.

9.2 Relationship of turnip rape and mustard allergy (III)

In study III we were able to show that also French children were sensitized to turnip rape and oilseed rape. Possible IgE cross-reactivity between turnip rape,

oilseed rape and mustard, all belonging to the same *Brassicaceae* family, was examined. SPT and IgE ImmunoCAP results revealed that 71% of the turnip rape-sensitized French children were sensitized to oilseed rape and 100% to mustard. Similar high frequencies were found in the Finnish children. Moreover, five (36%) of the turnip rape sensitized French children showed positive challenge reactions to turnip rape and another five to mustard. All 14 Finnish children showed positive challenge reactions to turnip rape and five (36%) to mustard.

Sensitization to turnip rape has not been studied previously in France, but several studies have shown that mustard allergy is common there (Rancé et al. 2000, Morisset et al. 2003a). This may not be unexpected, because France is the largest consumer of mustard in Europe and previously mustard was added even to baby foods (Rancé 2003). In contrast to France, in Finland the consumption of mustard is low and we are not aware of this spice being added to baby foods. It was therefore unexpected to find that all of the 14 Finnish children had positive SPT and all but one positive IgE ImmunoCAP to mustard. Moreover, the mustard challenge was positive in five (36%) Finnish children. This frequency was the same as in French children. In addition, IgE antibodies to the purified 2S albumins of these three seeds were measured by ELISA and the median IgE levels were found to be about the same in the Finnish and French children. ELISA inhibition studies by using IgE antibodies from the Finnish and French children confirmed that the 2S albumins of turnip rape, oilseed rape and mustard are highly cross-reactive. In addition to these plant seeds, 2S albumins have been shown to be allergens in sunflower seed, sesame seed and Brazil nut (Menéndez-Arias et al. 1988, Monsalve et al. 1993, Nordlee et al. 1996, Kelly et al. 2000, Pastorello et al. 2001, Beyer et al. 2002). Sesame sensitization and cross-reactivity seems, however, unlikely, because only one of the present 14 Finnish children showed a positive SPT to sesame seeds (unpublished data). Possible cross-reactivity between turnip rape, oilseed rape and mustard 2S albumin allergens and these 2S albumins in various other seeds and nuts needs to be studied in systematically selected series of sensitized patients by performing SPTs and cross-wise ELISA inhibition experiments with purified 2S albumins.

The challenge reactions to turnip rape differed significantly ($p < 0.001$) in the Finnish (100% positive) and French (36% positive) children in spite of identical reactions to mustard and no major differences in SPT and serum tests (III; Table 2). This may be due to the fact that the challenges were open and mostly done using a non-validated method (labial challenge). Mustard contains irritants such as capsaicin and isothiocyanates which is why labial challenges were not performed for mustard (Rancé 2003). To our knowledge, such irritants have not been described in turnip rape or oilseed rape. In addition, false positive irritant reactions in the labial turnip rape challenge were excluded by showing negative challenge reactions in 25 atopic control children in study I.

It is possible that turnip rape and oilseed rape rather than mustard are the main sensitizers, inducing IgE responses to 2S albumins of the *Brassicaceae* family in the present Finnish and French children. Rapeseed oil made from oilseed rape or turnip rape is commonly used in the food industry in Europe and elsewhere. According to food labels, rapeseed oil is added to margarine and even to baby foods, including milk formulas. It is not at present known whether rapeseed oil contains minute amounts of allergenic proteins, like refined and crude peanut, sunflower, sesame and soy oils do (Kanny et al. 1996, Hourihane et al. 1997, Teuber et al. 1997, Olszewski et al. 1998, Zitouni et al. 2000, Morisset et al. 2003b), and would thereby be capable of sensitizing children.

Study III showed that the young French children with AD, like the Finnish children, can be sensitized to turnip rape and oilseed rape. ELISA inhibition studies confirmed that the 2S albumin allergens in the seeds of the two oilseed plants and mustard are highly cross-reactive. Therefore, the 2S albumins of turnip rape, oilseed rape or mustard, or all three, could have been the primary sensitizers. Attention should be paid to the sensitization to these oilseed plants in the French children reactive to mustard and, on the other hand, to the possibility that a substantial number of the turnip rape- and oilseed rape-sensitized young Finnish children could be at risk of reacting to mustard.

9.3 Characterization of turnip rape and oilseed rape allergens (IV)

In study IV, IgE-binding allergens from the seeds of turnip rape and oilseed rape were identified as 2S albumins. Major allergenic proteins from turnip rape and oilseed rape were extracted and purified by gel filtration and cation exchange chromatography. Proteins in all the fractions obtained in the cation exchange chromatography showed similar immunoreactivity. IgE binding to purified proteins was very similar between these two oilseed plants and the different fractions. In IgE ELISA the majority (80%) of the 72 patients had positive reactions to all of the purified fractions from both plants. The identified allergens are 2S albumins that are water-soluble seed storage proteins. 2S albumins have been shown to be allergens, for example in mustard and sesame seed (Menéndez-Arias et al. 1988, Pastorello et al. 2001). Sesame sensitization and cross-reactivity seems, however, unlikely, because only one of the present 14 Finnish children showed a positive SPT to sesame seeds. On the other hand, 2S albumins of oilseed rape, turnip rape and mustard, all members of the *Brassicaceae* family, revealed a high sequence homology, implying that IgE cross-reactions are conceivable.

10. CONCLUSIONS AND FUTURE ASPECTS

The present studies show that 2S albumins in turnip rape and oilseed rape are new potential food allergens in children with AD in Finland as well as in France where cross-reactive mustard might be the primary sensitizer. Turnip rape and oilseed rape seeds are not consumed as such like sesame seeds or used in foods as a spice like mustard. Therefore, the source of the frequent sensitization observed in the Finnish children is a dilemma. One source could be vegetable oils. Seeds of turnip rape and oilseed rape are increasingly used for the production of these oils which are then used as such in food preparation or mixed in margarine, salad dressings and even in baby foods (Dupont et al. 1989). There is a need to perform detailed laboratory experiments and especially oral challenge studies with these oils to obtain firm evidence. Previous studies have shown that various crude and refined seed oils can contain IgE binding allergens and, importantly, food challenges with these oils have also been positive. For example, food challenge with refined peanut oil was positive in 23% of 62 sensitized French children (Moneret-Vautrin et al. 1998). In contrast, a randomized, double-blind study in 60 sensitized British adult patients did not find positive challenge reactions to refined peanut oil, but six (10%) patients reacted to the crude peanut oil (Hourihane et al. 1997). Other studies with refined peanut, sunflower and soy oils and with crude sesame oil have also shown positive challenge results (Olszewski et al. 1998, Morisset et al. 2003b, Leduc et al. 2006). When challenge-positive patients were skin prick-tested, only one had a positive SPT when refined peanut oil was tested as such, but several were positive to a protein fraction extracted from this oil (Moneret-Vautrin et al. 1998, Olszewski et al. 1998). We performed preliminary SPTs with crude and refined turnip rape oil as such in a few challenge-positive children, but the results were similarly negative. This evidence from other seed oils suggests that the rapeseed oil could also contain sufficient amounts of 2 S albumin allergens to cause

clinical symptoms in highly sensitized subjects. Supporting this, we have eliminated rapeseed oil from the diet of a few highly sensitized children (IgE ImmunoCAP to oilseed rape > 100 kU/l) and seen improvement of AD. This unpublished observation should be confirmed with a prospective elimination study which includes also double-blind rapeseed oil challenges.

We observed in our cohorts by skin prick testing and IgE antibody measurements high sensitization rates to turnip rape and oilseed rape. Whether the possible minute amounts of allergens in the rapeseed oil could have sensitized these infants and young children with AD is a further matter of discussion. The major 2S albumin allergens in the seeds of these plants contain oleosins which interact with phospholipids and participate in the formation and storage of oil bodies (Li et al. 1992, Olszewski et al. 1998, Leduc et al. 2006). The phospholipids are on the outer core of the oil bodies and the water-soluble albumins are in the inner core, due to which the 2S albumin allergens seem to be difficult to detect using conventional protein determination methods (Olszewski et al. 1998). Minute amounts of the 2S albumin allergens could be hidden inside the phospholipid core also in rapeseed oil and then after ingestion released in the gut. This could be one possible mechanism for the observed frequent sensitization which has been debated by one dietary lipid expert (Gylling 2006). In addition to rapeseed oil, cow's milk could also be a possible source of the frequent sensitization to turnip rape and oilseed rape. In Finland, a cow eats daily as much as three kilograms of protein-rich mass prepared from turnip rape and oilseed rape after the oil has been pressed out. Whether cow's milk could contain minute amounts of 2S albumin allergens from turnip rape and oilseed rape has not been established but should be studied. The same is true for human breast milk, because several reports have shown that infants on exclusive breast-feeding can be sensitized (Isolauri et al. 1999, Järvinen et al. 1999). In agreement with this, human breast milk can contain allergenic proteins such as cow's milk beta-lactoglobulin (Sorva et al. 1994). Mustard should also be kept in mind, because one study showed that cow's milk contained minute amounts of mustard flavoring ingredients when an enzyme-linked immunosorbent assay method was developed to detect traces of mustard protein (Koppelman and al. 2007).

In addition to the oral route, sensitization to turnip rape and oilseed rape through a respiratory route or the skin should be considered. Sensitization to oilseed rape pollen may rarely occur in an occupational setting and then the allergens are profilins and not the 2S albumins (Fell et al. 1992, Focke et al. 1998). There are a few reports on food allergic children who seem to have been sensitized to peanut and oats through the skin, e.g. from creams containing these allergens (Lack et al. 2003, Boussault et al. 2007). There is at least one cream on the market in Finland which contains rapeseed oil, but its use is minimal in children with AD and cannot explain the high sensitization rate to turnip rape and oilseed rape.

A large amount of work is needed before the source of sensitization to turnip rape and oilseed rape has been established. In the future, food challenges with rapeseed oil should be performed to assess the clinical relevancy of these oils in the induction of allergic reactions. In addition, animal and *in vitro* laboratory experiments would give a better understanding of the mechanisms of sensitization and identification of the allergen sources of turnip rape and oilseed rape. Further, a follow-up study in sensitized children with AD would give more information on the natural course of sensitization and allergy to these oilseed plants.

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Original article

Turnip rape and oilseed rape are new potential food allergens in children with atopic dermatitis

Background: When skin prick testing (SPT) young children with atopic dermatitis (AD) for suspected food allergy, we frequently found positive reactions with turnip rape (*Brassica rapa*) and oilseed rape (*Brassica napus*). We performed food challenge to examine whether these children react clinically to turnip rape.

Methods: A total of 1887 children were screened with SPTs for sensitization to turnip rape and oilseed rape. Twenty-eight children with clearly positive SPT (≥ 5 mm) were first subjected to labial challenge with turnip rape seeds followed, if negative, by open oral challenge for up to 7 days. Twenty-five children with AD but negative SPT to turnip rape and oilseed rape served as controls.

Results: Two-hundred and six (10.9%) children had positive SPT to turnip rape and/or oilseed rape. Twenty-five (89%) of 28 children showed a positive challenge reaction to turnip rape. Seventeen reacted with labial whealing, and eight in oral challenge with facial urticaria, flare-up of AD or abdominal symptoms. All 25 control children remained negative in the labial challenge.

Conclusions: Turnip rape and oilseed rape seem to be new important food allergens in young children with AD. The modes of exposure to these allergens and the possible routes of sensitization remain to be established.

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Key words: atopic dermatitis; food hypersensitivity; oilseed rape; turnip rape.

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In western countries young children suffer frequently from food allergy and are often sensitized to multiple foods (1). Cow's milk, egg, wheat, peanut, fish and soy are the main allergenic foods in these children who mostly present with atopic dermatitis (AD) (2, 3). At Tampere University Hospital, for over 10 years, we have routinely performed skin prick test (SPT) screening with a large selection of commonly used foods in infants and young children with AD referred for the investigation of food allergy. Mustard (*Sinapis alba*) has been found to cause food allergies, especially in France (4, 5), which prompted us to include mustard in our SPT series. As the rate of sensitization was high, we included turnip rape (*Brassica rapa*) and oilseed rape (*Brassica napus*), which belong to the same *Brassicaceae* family as mustard, in the SPT series and found again a constant high number of positive SPT reactions.

Allergy to pollens of oilseed rape may occur (6) but, to our knowledge, food allergy to turnip rape or oilseed rape has not been previously described. Both plants are widely used in vegetable oil production. Oilseed rape ranks as the

most commonly grown oilseed crop in Europe and turnip rape is particularly used by Finnish food industry (7).

In the present study we investigated by open food challenge with seeds of turnip rape whether children with positive SPTs to oilseed rape and turnip rape are also clinically reactive to these plants.

Methods

Patients

A total of 1887 children under 16 years of age (781 girls and 1106 boys) were screened with turnip rape and oilseed rape SPT during a 2-year period from April 2002 to April 2004. They all had been referred to the Department of Dermatology at Tampere University Hospital for evaluation of food allergy. In these children SPT were positive to cow's milk in 19%, to wheat in 20%, to egg in 31% and to birch pollen in 10%. Most of these children presented with AD and 1337 (70%) of them were below 3 years of age.

Inclusion criteria for the challenge study with turnip rape were that the sensitized children showed in SPT a wheal with a mean diameter of ≥ 5 mm to turnip rape or oilseed rape. Twenty-eight children (mean age 4.8 years), whose demographic and clinical characteristics are shown in Table 1, were enrolled in the challenge study. Seventeen of them were allergic to cow's milk and 24 to wheat, as confirmed by open food challenges. The Ethical

Abbreviations: AD, atopic dermatitis; SCORAD, AD symptom score; SPT, skin prick test.

Table 1. Demographic and clinical characteristics of the 28 children with positive skin prick test (SPT) reactions to turnip rape and oilseed rape participating in the turnip rape challenge study

Variables	Value
Age, mean (range), years	4.8 (1.0–15.4)
Exclusive breastfeeding, median (range), months	4.0 (1.0–5.5)
Atopic heredity: parent or sibling, <i>n</i> (%)	26 (93)
Personal atopic disorder	
Atopic dermatitis, <i>n</i> (%)	28 (100)
SCORAD, mean (range)	26 (9–60)
Asthma, <i>n</i> (%)	18 (72)
Food allergy	
Milk, <i>n</i> (%)	17 (61)
Wheat, <i>n</i> (%)	24 (86)
Egg*, <i>n</i> (%)	26 (93)
Total IgE, median (range), kU/l	1150 (26 to >10 000)
Sensitization to inhalant allergens	
Mugwort, <i>n</i> (%)	15 (54)
Timothy, <i>n</i> (%)	17 (61)
Birch, <i>n</i> (%)	23 (82)

*Based on symptoms and positive SPT and/or specific IgE.

Committee of Tampere University Hospital approved the challenge study and informed consent was obtained from the parents.

Skin prick tests

Skin prick testing was performed with crushed seeds of turnip rape (*B. rapa* ssp. *oleifera*) and oilseed rape (*B. napus* ssp. *oleifera*) moistened with physiological saline using a commercial one-peak lancet (ALK-Abelló A/S, Hørsholm, Denmark) and prick-prick method (8). The seeds were obtained from a local manufacturer of turnip rape oil (Mildola oy, Kirkkonummi, Finland). Histamine dihydrochloride (10 mg/ml; ALK-Abelló) was used as a positive and saline (Soluprick SQ; ALK-Abelló) as a negative control.

IgE antibodies to oilseed rape

The IgE antibodies to oilseed rape were measured using ImmunoCAP (Pharmacia CAP System Specific IgE RIA, Uppsala, Sweden) in 28 challenged children. The sera were stored at –20°C before testing. Values ≥0.4 kU/l were considered positive.

Labial and oral challenge with turnip rape

The turnip rape challenge was open and started with the application of a small amount of oily mass of crushed seeds of turnip rape on the lip (9, 10). If the labial challenge was negative, increasing doses of crushed seeds of turnip rape (10, 10, 30, 100, 300 and 900 mg) were given orally every 20 min. Appearance of wheals or labial swelling or both were regarded as positive. Oral challenges were carried out with turnip rape seeds mixed in an appropriate food tolerated by the child. If negative on the first day, the challenge was continued at home for 6 days by giving 1350 mg of turnip rape daily. The severity of AD was scored at the beginning and at the end of the challenge by SCORAD (11). All gastrointestinal and respiratory symptoms were also registered during the challenge. False-positive irritant reactions in the labial challenge were excluded by challenging lips of 25 children (10 girls and 15 boys) with AD under 10 years of age, but with negative SPT to turnip rape and oilseed rape. All controls had AD, 60% had allergy to cow's milk and 48% to wheat confirmed by food challenge. In addition 60% were sensitized to egg.

Statistical analysis

Statistical comparison between the groups was made by using permutation test. Correlation coefficients were calculated by the Spearman method.

Results

Two hundred and six (10.9%) of the 1887 children screened had a clearly positive (≥5 mm) SPT to turnip rape (9.3%) or oilseed rape (9.4%). Eleven percent (149/1337) of the children under 3 years of age were sensitized to turnip rape and/or oilseed rape.

Twenty-five (89%) of the 28 children had a positive and three (11%) had a negative turnip rape challenge (Table 2). The labial challenge was positive in 17 (68%) children showing whealing and/or swelling. In addition, one child got rhinitis. The oral challenge was positive in eight children. Four of the children got immediate symptoms within <3 h and four others during the challenges at home. All 25 control children remained negative in the turnip rape labial challenge.

Table 2. Clinical types of challenge reactions to turnip rape in 25 children

	Positive challenge tests to turnip rape		
	Labial (<i>n</i> = 17)	Immediate oral (<i>n</i> = 4)	Delayed oral (<i>n</i> = 4)
Age, years, mean (range)	4 (1–9)	4 (1–10)	8 (5–15)
Oilseed rape ImmunoCAP (kU/l), median (range)	21.6 (0.70 to >100)	23.5 (2.19 to >100)	7.3 (0.90–16.30)
Skin prick test, wheal diameter (mm)			
Turnip rape, median (range)	8 (5–23)	7 (6–9)	5 (4–8)
Oilseed rape, median (range)	7 (5–25)	7 (4–12)	5 (4–7)
Challenge			
Dose (mg), median (range)	Small amount	500 (20–1350)	2700 (2700–6750)
Symptoms, <i>n</i>	Labial urticaria, 17 + rhinitis, 1	Facial urticaria, 2 Generalised erythema, 1 Abdominal complains, 1	Flare up of AD, 3* Flare up of asthma and abdominal complains, 1

*SCORAD mean (range) change: 24 (11–53) to 31 (11–62).

There was a clear correlation between the size of turnip rape and oilseed rape SPTs [0.69 (95% CI: 0.43–0.95)] in the 28 children challenged with turnip rape (Fig. 1). The SPT results to turnip rape and IgE antibody levels to oilseed rape in challenge-positive and challenge-negative children are given in Fig. 2A and 2B. At the time of challenge SPT reaction to turnip rape and oilseed rape had decreased < 5 mm in two children, whereas in all the

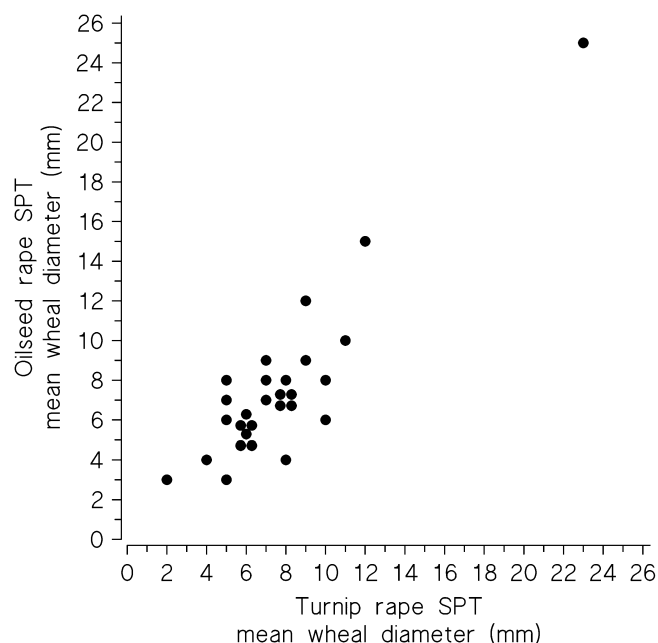


Figure 1. Skin prick test (SPT) reactivity to oilseed rape and turnip rape shows a significant [0.69 (95% CI: 0.43–0.95)] correlation in the 28 challenged children.

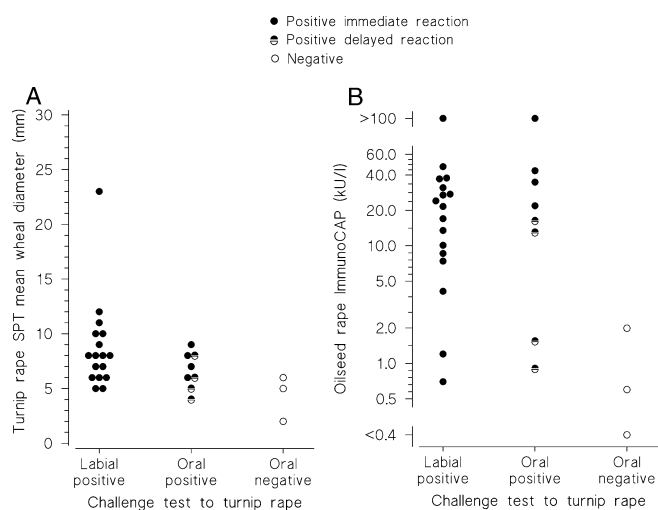


Figure 2. (A) Skin prick test (SPT) reactivity and challenge test; (B) IgE antibody levels to oilseed rape and challenge reactions to turnip rape in 28 children.

other children SPT was still clearly positive. The first child with a small SPT reaction showed flare up of AD, whereas in the second child the turnip rape challenge was negative. Only one of the 25 control children had IgE antibodies (2.1 kU/l) to oilseed rape. The total IgE was 1510 kU/l (median; 95% CI: 634–1790 kU/l) in the 25 challenge-positive children. In the three challenge-negative children, the total IgE was 26, 27 and 323 kU/l, respectively.

Discussion

In the present study we found as high a prevalence as 11% of positive SPT reactions to turnip rape or oilseed rape in a cohort of 1887 Finnish children examined for suspected food allergy. Twenty-eight children with AD and positive SPTs to turnip rape and oilseed rape and 25 control children with AD and negative SPTs, respectively, participated in the open turnip rape challenge study. Most (89%) of the sensitized children showed positive labial or oral challenge reactions to turnip rape in contrast to none of the SPT-negative control children. The challenge reactions were mostly of immediate type such as labial swelling or facial urticaria. The dose of crushed turnip rape seeds causing positive oral challenge varied from 20 to 1350 mg. The large variation in the cumulative reactive doses correlates well with previous results in peanut and sesame seed challenge studies (12). Three challenge-positive children showed flare up of AD when the oral challenge had lasted for 2–5 days. Similar delayed type challenge reactions have been observed previously with cow's milk and wheat (13–15). When the 25 challenge-positive and three challenge-negative children were compared, the latter had smaller SPT reactions to turnip rape and oilseed rape and also lower IgE antibody levels to oilseed rape, suggesting a lower sensitization level to these plants. There was a tendency that younger children showed larger SPT reactions and immediate type responses to smaller amount of turnip rape seeds than older children, but the numbers of children in the variously reacting challenge groups were small.

Positive labial or oral challenges with turnip rape in the majority of the infants and young children with IgE antibodies to oilseed rape suggest that both of these oilseed plants could be clinically relevant food allergens. The observed high correlation in the SPT reactivity between oilseed rape and turnip rape suggests allergic cross-reactivity, supported also by recent finding of highly homologous 2S albumin allergens in both of them (T.J. Puumalainen, unpublished data). Interestingly, in France mustard allergy is now ranked fourth in children's food allergies (4) and a 2S albumin has been identified as a major allergen in mustard (*Sinapis alba*, *Brassica juncea*) (16, 17). Allergy to sesame (*Sesamum indicum*) has been reported to occur, e.g. in Israel and France and

also seeds of this plant contain allergens belonging to the 2S albumin family (18, 19). Even low-grade consumption of mustard or sesame by the Finnish infants and young children seems unlikely because to our knowledge these agents are not added to commercial or home-made baby or children's food. In contrast, turnip oil is commonly used in the Finnish food industry and is included, e.g. in margarine and baby food (20). However, it remains to be settled whether biologically meaningful amounts of allergenic proteins are present in turnip rape or oilseed rape oils. In the present study we performed preliminary SPTs with refined and cold-pressed turnip oil in a few of the challenge-positive children, but all SPTs were negative (data not shown). It may be noted that cold-pressed and refined oils, such as peanut and sesame oil, have been

reported to cause allergic reactions in highly sensitized individuals (12, 21), which implies that extended studies in this field are warranted.

In conclusion, turnip rape and oilseed rape are potentially important allergens in infants and young children. An oral route seems obvious for sensitization, but the food sources and clinical consequences of this new allergy are challenges for future research.

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