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# Lupin allergy and lupin sensitization among patients with suspected food allergy

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**Background:** Lupin, a legume with good nutritional value, is used in food production today, most often in bakery products. Lupin sensitization is often seen among patients with reactions to legumes, but the number of reports describing lupin anaphylaxis is also increasing.

**Objective:** To investigate the occurrence of lupin sensitization, cross-reactivity, and lupin allergy among patients with suspected food allergy in Finland, where lupin is a labeled ingredient in few products.

**Methods:** The occurrence of positive skin prick test (SPT) reactions to lupin seed flour was studied among 1,522 patients with suspected food allergy from November 1, 2005, through December 31, 2007. Clinical histories and diagnostic SPT results were analyzed among patients with positive SPT results to lupin. For 1 patient, ImmunoSpot and lupin radioallergosorbent test inhibition methods were used.

**Results:** Lupin sensitization was shown in 25 of 1,522 patients (1.6%), and probable lupin allergy was diagnosed in 7 of 25 patients, in whom the clinical symptoms varied from anaphylaxis and respiratory symptoms to contact urticaria and itchy mouth. Cross-reactions or concurrent reactions to other legumes were seen in 18 of 25 patients.

**Conclusions:** Clinically relevant lupin allergy often occurs in patients without atopic background or other food allergies, although lupin sensitization most commonly seems to represent cross-reactivity to other legumes. The occurrence of lupin allergy in a country where lupin has not been traditionally used is surprisingly common, suggesting that short-term use of modest amounts of lupin can cause serious allergic reactions.

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## INTRODUCTION

Lupin belongs to the legume family along with soy, pea, peanut, lentils, and beans. Lupin is cultivated around the world for animal and human use. White lupin (*Lupinus alba*), yellow lupin (*Lupinus lutes*), and blue lupin (*Lupinus angustifolius*) seeds are high in protein and dietary fiber content. Lupin flour can be used in much the same way as soy flour. It is used in bakery products and other food to improve the nutritional value. In Europe the consumption of lupin products is most extensive in France and Mediterranean countries. In the European Union (EU) area lupin belongs to the useful plants that receive farming subsidy.

Lupin allergy has been reported to cause urticaria,<sup>1</sup> asthma,<sup>2</sup> angioedema,<sup>1,3</sup> anaphylaxis,<sup>1,4</sup> and contact urticaria.<sup>5</sup> Lupin may be hidden in various food products. In December 2006 the European Commission (EC) submitted directive 2006/142/EU. It amends the EU foodstuff allergen list to include lupin.<sup>6</sup>

As a member of the legume family, lupin has been shown to have allergenic cross-reactivity with peanut, soy, and

pea.<sup>7,8</sup> Lupin allergy seems to occur in atopic individuals with sensitization to multiple legumes,<sup>9</sup> as well as in individuals without reactivity to other legumes.<sup>10</sup>

In Turku and Tampere University hospitals, skin prick tests (SPTs) with lupin flour have been performed among patients studied for suspected food allergy since the end of 2005. In this study the occurrence of positive SPT reactions to lupin flour, associated reactions, and allergy symptoms are described.

## METHODS

### Patients

From November 1, 2005, through December 31, 2007, SPTs with lupin flour were performed on 1,522 patients with symptoms suspected to be derived from food allergy (eg, urticaria, worsening of atopic dermatitis, and respiratory symptoms) at the Departments of Dermatology in Turku and Tampere University Hospitals. Institutional review board approval was not required because the SPTs, including lupin flour, were performed as part of a normal medical examination. For the same reason, no informed consent was needed from the patients. The history and symptoms of the patients with positive SPT reactions to lupin are given in Table 1. In addition, 1 patient diagnosed as having lupin allergy before the study period is introduced.

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Table 1. History and Symptoms of Patients With Positive Skin Prick Test Reactions to Lupin

Patient No./sex/age, y	Atopy	Exposure to lupine	Current symptoms	Ctl	L	P	S	Pe	T	H	M	SF
1/F/39	AR, AE	Unknown	Angioedema	3	5	0	12	4	17	0	0	0
2/F/18	AR, A	Unknown	Oral symptoms	5	6	0	10	ND	20	18	0	6
3/F/45	AR	Unknown	Anaphylactic reaction	5	5	0	6	ND	13	4	3	8
4/M/17	AE, AR	Unknown	Angioedema	3	4	4	5	4	7	5	0	6
5/M/8	AE, AR	Unknown	Urticaria, angioedema	4	4	14	9	7	4	ND	ND	5
6/F/38	AE, AR	Unknown	AE	5	5	7	9	4	8	12	8	3
7/M/2	AE, A	Unknown	AE	5	7	ND	5	ND	5	5	0	7
8/M/30	AE, A, AR	Unknown	AE	4	4	12	0	3	5	4	3	6
9/M/12	AE, AR	Unknown	AE	5	5	5	5	4	5	7	3	6
10/M/28	AR	Unknown	Urticaria, angioedema	4	7	0	3	5	6	7	0	6
11/F/45	AR	Unknown	Urticaria	6	6	6	6	7	8	6	8	5
12/M/17	AR	Unknown	Urticaria, angioedema	7	10	10	6	5	3	13	7	8
13/F/23	AE	Unknown	AE	5	5	5	4	4	0	0	0	3
14/M/5	AE	Unknown	AE	4	10	8	0	0	3	ND	ND	ND
15/F/0.7	AE	Unknown	AE	5	7	8	3	ND	0	ND	ND	ND
16/M/20	AE, AR	Unknown	AE, AR	4	8	6	9	0	6	7	0	6
17/M/19	AE, AR, A	Unknown	Oral symptoms	4	9	6	26	3	8	8	4	16
18/F/59	AE, AR	Unknown	AE	5	6	0	5	0	8	3	12	11
19/M/15	AE	Handling of forage	AE	4	7	0	0	0	4	11	0	5
20/F/39	No	Lupin in biscuits	Angioedema	7	11	0	0	0	0	0	0	0
21/M/42	No	Bakery flour	Rhinitis, dyspnea	7	11	0	0	0	0	0	0	0
22/F/36	No	Health food product	Anaphylactic reaction	5	10	0	0	0	0	0	0	0
23/M/20	No	Hot dog	Anaphylactic reaction	4	15	0	0	3	0	0	0	0
24/F/53	No	Karelian pastry	Angioedema	5	11	0	4	0	0	4	0	0
25/M/13	AE, AR	Imported biscuits	AE, oral symptoms	4	15	15	3	4	ND	ND	ND	3

Abbreviations: A, asthma; AE, atopic eczema; AR, allergic rhinitis; Ctl, positive control; H, hay; L, lupin; M, mugwort; ND, not done; P, peanut; Pe, pea; S, soy bean and/or flour; T, deciduous tree; SF, sunflower seed.

### Skin Prick Tests

The patients were tested with the standard SPT series of pollen (birch, alder, timothy, mugwort), animals (horse, cat, dog), and a series of commercial foods (green pea, peanut, hazelnut, and almond) (Soluprick SQ allergens; ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark). By using a 1-peak lancet (ALK) and the prick-to-prick method, SPTs were also performed with fresh fruits (apple, avocado, and mango), roots (carrot, potato, and parsnip), crushed soy beans moistened with saline, and crushed sunflower seed moistened correspondingly. Because of the lack of commercial skin test extracts, lupin seed flour (purchased from Hurme Yhtiöt, Turku, Finland) and soy flour, both mixed with physiologic saline (1:9), were tested by using the skin prick-to-prick method. Histamine dihydrochloride (10 mg/mL; ALK) was used as a positive control and saline (Soluprick; ALK) as a negative control. The largest diameter and the diameter opposite it were measured at 15 minutes. A reaction was interpreted as a positive reaction when the mean of the wheal diameters was equal to or more than that of the positive control. There were also 3 patients in whom the mean of the wheal diameter was 3 mm or greater, but smaller than the positive control test result. All of these patients also had several other positive SPT reactions and no history of lupin consumption. Therefore, these patients were left out of this study.

### In Vitro Tests

Serum for IgE tests was available from 1 patient only (patient 20). Serum lupin specific IgE was measured in CAP-FEIA (Phadia, Uppsala, Sweden) in 2 patients. In addition, an in-house ImmunoSpot method was used to evaluate IgE antibodies to a biscuit causing anaphylaxis in patient 20.<sup>11</sup> A wide range of other allergens were evaluated in the same assay, for example, legumes (peanut, soy, pea, guar gum), tree nuts (hazel nut, walnut, almond, cashew), grains (wheat, gliadin, corn, corn starch), cod, egg white, milk, latex, pollens (birch, mugwort, timothy), animals (cat, dog, horse), mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*), and molds (*Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*). Nonatopic serum and a pool of atopic sera were used as control sera. Allergen cross-reactivity between lupin and peanut, soy, and pea was evaluated in lupin radioallergosorbent test (RAST) inhibition<sup>11</sup> in which also the lupin allergenicity of the anaphylactic biscuit was evaluated.

### RESULTS

Positive SPT reactions were seen in 25 of 1,522 patients (1.6%) (Table 1). The age of these patients varied from 8 months to 59 years. Eleven patients had a history of urticaria, angioedema, or an anaphylactic reaction, and 3 patients had experienced oral symptoms. Food ingestion was suspected to

cause deterioration of eczema in 11 patients. One bakery worker had rhinitis and dyspnea.

Concomitant positive SPT reactions to other legumes (soy, peanut, and/or pea) were seen in 18 of 25 patients, of whom 13 reacted to peanut and pea and 18 to soy. Three children (8 months, 2 years, and 5 years) also had milk and egg allergy, and they also reacted to other legumes.

Positive SPT reactions to tree, hay, and mugwort pollen were seen in 19, 15, and 15 of 25 patients, respectively (Table 1). Reactions to almond and/or hazelnut were seen in 14 and to fruits and/or roots in 9 pollen sensitized patients.

Seven of the 25 SPT-positive patients were regarded as having clinically relevant lupin allergy. An atopic background was apparent in 2 of them based on clinical symptoms (atopic eczema and, for the other, also allergic rhinitis). One of them used to work in a farm (patient 19). He had experienced intensive itching, redness, and urticaria of the arms elicited by lupin during forage in the beginning of the summer. Another atopic patient had a history of oral symptoms when eating imported biscuits also containing lupin (patient 25).

#### Lupin Allergy in Nonatopic Patients

Clinically relevant lupin allergy was proven in 2 patients (patients 20 and 22) and probable in another 3 (patients 21, 23, and 24).

#### Patient 20

In April 2005, a 39-year-old woman with a history of 2 episodes of suspected angioedema related to food ingestion was referred for treatment. In her youth she had been diagnosed as having celiac disease and lactose intolerance. No allergies had been diagnosed previously. She had experienced burning and tingling in her mouth and swelling of the tongue when eating gluten-free biscuits.

When the patient arrived for the SPTs lupin flour was not available for skin testing, because lupin allergy was not yet known in our hospitals. Results of a SPT with a 10% extract of the suspected biscuits in saline were strongly positive (11 mm with pseudopods). The patient felt stinging and swelling in the throat after the skin testing. She was treated with antihistamine, adrenalin, methylprednisolone, and theophylline.

In vitro studies showed a high serum IgE antibody value to lupin (85.6 kU/L). A distinct IgE binding to suspected biscuits, lupin seed, and lupin flour was seen in ImmunoSpot (Fig 1). There was a weak, nonspecific binding to peanut, soy, pea, and wheat but not to gliadin or the other allergens tested. There was no distinct binding to biscuit or lupin from the nonatopic and atopic control sera. In specificity studies using the RAST inhibition method, a significant inhibition of the IgE binding to solid phase lupin was demonstrated with the biscuit extract (95% inhibition) and lupin seeds (73% inhibition), but not with other legumes (1% to 11% inhibition) (Fig 2).

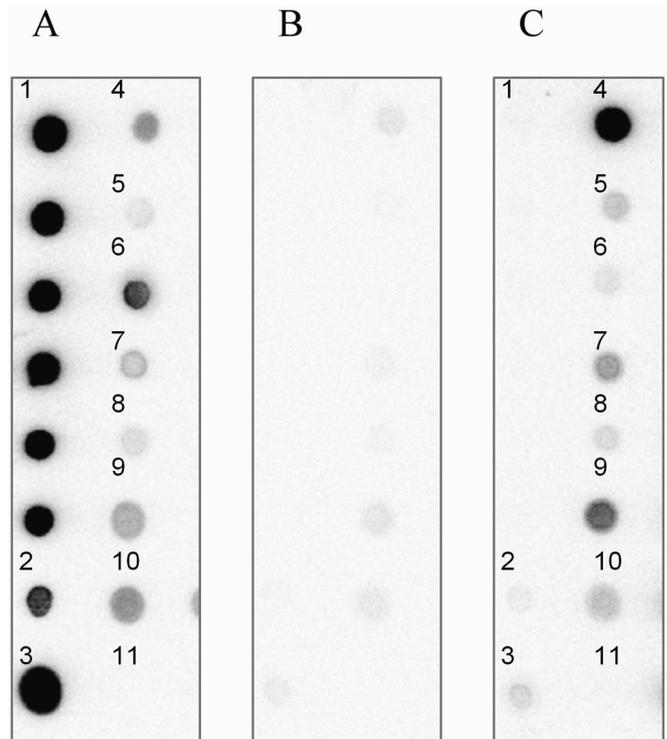


Figure 1. Specific IgE binding in ImmunoSpot. IgE binding was measured by ImmunoSpot from patient (patient 20) (A), nonatopic (B), and atopic (C) control sera. Allergen samples are biscuit (1), lupin flour (2), lupin seed (3), peanut (4), hazelnut (5), walnut (6), almond (7), cashew (8), soy (9), pea (10), and guar gum (11).

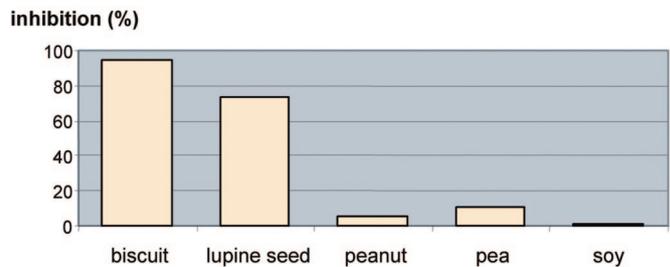


Figure 2. Specific IgE binding in lupin radioallergosorbent test (RAST) inhibition. The RAST inhibition study was performed with serum from patient 20. Inhibitors are biscuit, lupin seed, peanut, and soy. Only the biscuit and lupin showed a significant inhibition of more than 20%.

#### Patient 21

A 42-year-old male bakery worker had experienced work-related rhinitis, sneezing, and dyspnea during 1.5 years. Pulmonary function test results were within normal limits. The SPT result with lupin was 11 mm. The SPT results with soy, peanut, pea, and flour used in the bakery were negative. The IgE antibody test (CAP-FEIA; Pharmacia) result against lupin seed was 3.5 kU/L, and the total IgE level was normal (41 kU/L). Because the bakery was also using imported flour, it

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was presumed that some batches contained or had contained lupin flour. All the symptoms subsided within 2 years.

#### *Patient 22*

A 36-year-old woman bought a health food powder with multiple ingredients that contained 60% lupin flour. The powder was ingested mixed in water. The patient had enjoyed this product weekly during 1½ months before she started to experience dyspnea with angioedema, leading to anaphylaxis after ingestion of this product. The results of SPTs with lupin and the product were positive. The other ingredients of the lupin product were tested separately, and no reactions were seen to ingredients other than lupin.

#### *Patient 23*

A 20-year-old man without any history of previous allergy symptoms experienced angioedema, dyspnea, and an anaphylactic reaction after eating fast food-type meat pastry and tomato ketchup. The results of SPT to lupin were positive without other significant SPT reactions. Lupin was not listed among the ingredients of the pastry or the ketchup, but imported flours were used in bakery, where the pastry was produced. Lupin contamination in the pastry was suspected.

#### *Patient 24*

A 53-year-old woman had experienced an anaphylactic reaction 3 times after eating Karelian pastries produced by a small local bakery. The ingredients of the pastries could not be confirmed. A few weeks after the positive SPT result to lupin, the patient reported having knowingly tasted a lupin-containing biscuit, which caused swelling in the lips and tongue. Lupin contamination was suspected in the flours of the Karelian pastries.

## **DISCUSSION**

The occurrence of lupin sensitization was 1.6% among patients tested for suspected food allergy. In Portugal, 4.1% of the patients had positive SPT results to lupin.<sup>12</sup> Difference in the sensitization rates is expected because lupin consumption is more common in the Mediterranean countries. During the study period positive SPT reactions to peanut were seen in 8.2% and to soy in 11.4% of all adult (mainly atopic) patients tested for suspected food allergy, and a few of those patients also had a positive SPT result to lupin. In a recent report from France, the peanut sensitization rate was 7.2% among patients with an ongoing atopic disease.<sup>13</sup> Because an almost similar peanut sensitization rate seems to occur in Finland and in other European countries, food composition also appears to be important.

Among our patients cross-reactivity with legumes seems to explain the positive SPT reaction to lupin in most patients (76%) because there is no history of lupin ingestion. It is, however, also possible, that these patients have ingested lupin not knowing it. Peanut allergens are, to some extent, similar to those in lupin,<sup>14</sup> and in this study half the lupin-sensitized patients were also sensitized to peanut. In a recent report 34% of patients with peanut allergy were also sensitized to lupin,

but only 4% of the patients with peanut allergy had clinical lupin allergy when orally challenged.<sup>15</sup> In another recent study, a positive challenge reaction to lupin was seen in only 1 of the 10 challenged children with positive SPT reactions to lupin.<sup>16</sup> In our study oral challenge was not performed, but in 2 patients the association between lupin ingestion and angioedema was apparent (patients 20 and 22). Another peanut allergic patient had a history of lupin ingestion-associated symptoms (patient 25), and 1 had performed a mucosal challenge at home (patient 24).

Among our patients lupin sensitization was seen even in an 8-month-old child. Besides a positive SPT reaction to lupin, she had a similar reaction to peanut and a smaller reaction to soy. This child had not eaten peanuts. It has been reported that if the mother has eaten peanuts frequently during pregnancy, the child seems to have an increased risk of peanut sensitization.<sup>17</sup> It is not known whether lupin allergy can also develop during pregnancy if the mother eats lupin.

A positive SPT reaction to soy was seen in 6 patients without reactions to peanut. Soy sensitization was seen in 18 of 25 of those with a positive SPT reaction to lupin. Soy has been regarded as a cause of severe allergy reactions more seldom than peanut.<sup>18</sup> Among these lupin-sensitized patients, a history of soy ingestion-associated allergy symptoms (discomfort in the mouth, urticaria, angioedema, or anaphylaxis) was occasionally reported. One patient with evident primary lupin sensitization and lupin allergy had a smaller reaction to soy (patient 24). ImmunoSpot study could have been helpful with this patient. With patient 20 ImmunoSpot revealed non-specific binding with peanut, soy, and pea, whereas distinct binding with lupin products were seen. Lupin allergy has been reported to develop by sensitization to unique lupin allergens,<sup>14,19</sup> and even sensitization via respiratory route leading to rhinitis and/or asthma has been described earlier.<sup>4,20</sup> In this material, occupational sensitization and allergic rhinitis in 1 patient remained unproven.

Clinically relevant lupin allergy is not frequent in patients with positive SPT reactions to both lupin and other legumes. However, ingestion of 100 mg of lupin flour has been described to elicit allergy symptoms in such patients.<sup>8</sup> Even anaphylaxis caused by lupin has been described in a patient with primary sensitization to peanut.<sup>3</sup> Thus, those lupin-sensitized patients without any history of lupin-induced symptoms also need information concerning the occurrence of lupin in foods.

In 2 of our patients with severe anaphylaxis a hidden source of lupin in bakery products was regarded as the most apparent cause of anaphylaxis. Food contamination with small amounts of lupin probably occurs. Lupin allergens are resistant to normal cooking procedures, including boiling and microwave heating.<sup>21</sup> A novel sandwich enzyme-linked immunosorbent assay for the detection and quantification of lupin in processed foods using a polyclonal rabbit antilupin antibody has been developed.<sup>22</sup> Using immunological and polymerase chain reaction methods, lupin proteins have been detected in food products without lupin declaration (eg, in

bread and soy flour).<sup>23,24</sup> Our study suggests that a risk for anaphylaxis exists also after primary sensitization to lupin proteins, and in these cases unique lupin protein allergens seem to cause anaphylaxis with a minimal threshold dose. Recently, conglutin  $\beta$  has been identified as a specific *L. angustifolius* and *L. albus* allergen, designated Lup an 1.<sup>25</sup> Anaphylactic reactions to lupin flour in patients with no clinically relevant previous allergies or atopic diseases have also been reported previously.<sup>26,27</sup> Today EU directive 2006/142/EC amends the EU foodstuff allergen list to include lupin. By the end of the transition period (until the end of the year 2008), lupin has to be labeled in all products. By labeling and analyzing the lupin contamination in foods, some cases of serious allergic reactions could be avoided in the future.

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