

Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results

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Background: The hazelnut major allergens identified to date are an 18-kd protein homologous to Bet v 1 and a 14-kd allergen homologous to Bet v 2. No studies have reported hazelnut allergens recognized in patients with positive double-blind, placebo-controlled food challenge (DBPCFC) results or in patients allergic to hazelnut but not to birch.

Objective: We characterized the hazelnut allergens by studying the IgE reactivity of 65 patients with positive DBPCFC results and 7 patients with severe anaphylaxis to hazelnut.

Methods: Hazelnut allergens were identified by means of SDS-PAGE and IgE immunoblotting. Further characterization was done with amino acid sequencing, evaluation of the IgE-binding properties of raw and roasted hazelnut with enzyme allergosorbent test inhibition, assessment of cross-reactivity with different allergens by means of immunoblotting inhibition, and purification by means of HPLC.

Results: All the sera from the patients with positive DBPCFC results recognized an 18- and a 47-kd allergen; other major allergens were at molecular weights of 32 and 35 kd. Binding to the 18-kd band was inhibited by birch extract, indicating its homology with the birch major allergen, and abolished in roasted hazelnut. The 47-kd allergen is a sucrose-binding protein, the 35-kd allergen is a legumin, and the 32-kd allergen is a 2S albumin. Patients with severe anaphylactic reactions to hazelnut

showed specific IgE reactivity to a 9-kd allergen, totally inhibited by purified peach lipid-transfer protein (LTP), which was heat stable and, when purified, corresponded to an LTP.

Conclusions: The major allergen of hazelnut is an 18-kd protein homologous to Bet v 1, and the 9-kd allergen is presumably an LTP. Other major allergens have molecular weights of 47, 32, and 35 kd. (*J Allergy Clin Immunol* 2002;109:563-70.)

Key words: Food allergy, hazelnut, immunoblotting, major allergen

Hazelnut is a common allergenic food often implicated in severe anaphylactic reactions, as reported in several studies.¹⁻⁵ It belongs to the peanut and tree nut group, including walnut, almond, cashew, and Brazil nut, which, although botanically unrelated, are often the cause of clinically associated reactions. In the United States peanut and tree nut allergy affects about 1.1% of the population.⁶ Thus efficient diagnostic methods are needed for these foods to properly identify subjects at risk.

The lack of standardization of commercial food extracts for allergy tests, mainly the result of incomplete knowledge of their main allergens, results in low diagnostic accuracy for many foods,^{7,8} including hazelnut, as we recently demonstrated.⁹ Thus proper identification of the major allergens would be expected to lead to significant improvement in diagnosis. This can be achieved by studying the pattern of IgE reactivity against a certain food with sera from a large number of patients with clinically demonstrated reactions toward that food.

Few investigations have aimed at identifying hazelnut allergens. Hirschwengler et al¹⁰ studied sera from patients allergic to hazel pollen with hazelnut allergy and identified the major IgE-binding proteins of hazelnut as an 18-kd allergen similar to Cor a 1 and a 14-kd allergen similar to Cor a 2. Another study demonstrating in vitro cross-reactivity between hazelnut extract and mugwort pollen showed allergens with molecular weights of 17, 42, and less than 16 kd in hazelnut, without identifying the mugwort homologous allergens.¹¹

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
EAST: Enzyme allergosorbent test
LTP: Lipid-transfer protein

Recently, we conducted a multicenter clinical study to investigate clinical and immunologic reactivity to hazelnut.⁹ Sixty-seven patients were selected on the basis of a positive double-blind, placebo-controlled food challenge (DBPCFC) result to hazelnut and tested with the same method in 3 centers in Copenhagen, Milan, and Zurich.

Thus the present study sought to identify hazelnut allergens by analyzing a large number of sera from patients of different origin with documented clinical reactions. Sera of 7 patients with severe allergic reactions to hazelnut but no allergy to birch were also analyzed to check their IgE reactivity pattern.

METHODS**Study population**

This study used the sera of 65 of 67 patients with a positive DBPCFC result for hazelnut who were recruited in a previous study (Fig 1).⁹ They all presented with oral allergy syndrome, but 59 patients had oral allergy syndrome localized to the oral cavity, 3 had oral and gastrointestinal reactions, and 5 had oral and mild systemic symptoms. In Milan the sera of 7 additional patients (Fig 2) with a history of severe anaphylaxis to hazelnut were analyzed separately to evaluate their IgE reactivity patterns. Only one was clinically allergic to birch.

Determination of allergen-specific IgE antibodies

CAP-RAST for hazelnut, Bet v 1, and Bet v 2. IgE-specific antibodies for hazelnut and the birch allergens Bet v 1 and Bet v 2 were determined according to the manufacturer's instructions (Pharmacia & Upjohn, Uppsala, Sweden). The results were expressed in classes of positivity from 0 to 6, where class 0 corresponds to less than 0.35 kU/L, class 1 to 0.35 to 0.7 kU/L, class 2 to 0.7 to 3.5 kU/L, class 3 to 3.5 to 17.5 kU/L, class 4 to 17.5 to 50 kU/L, class 5 to 50 to 100 kU/L, and class 6 to greater than 100 kU/L.

Enzyme allergosorbent test for raw and roasted hazelnut. For additional IgE determinations, extracts from raw or roasted hazelnut (140°C for 40 minutes) were coupled to cyanogen bromide-activated filter-paper disks (Hycor, Kassel, Germany) at optimized concentrations of 50 µg of total protein per disk. Enzyme allergosorbent tests (EASTs) were performed with Allergopharma Spez. IgE ELISA (RV), according to the manufacturer's instructions (Allergopharma, Reinbek, Germany). Results were expressed as EAST classes 0 to 4, corresponding to the arbitrary RAST units, with class 4 being 17.5 kU/L or greater.

Allergen extracts

Raw hazelnut extract was prepared as follows. Raw nuts of the Piemonte variety, ground in a mixer, were defatted by means of several passages in acetone, followed by paper filtration, and then dried overnight. The powder was suspended 1:10 (wt/vol) in 0.1 mol/L PBS (pH 7.4) for 2 hours at 4°C to 8°C. The protein concentration of the extract was determined with Folin reagent, according to the method of Lowry et al.¹²

Extract from roasted hazelnuts was prepared as described elsewhere.¹³ Birch pollen extract was supplied by Pharmacia & Upjohn. Peach extract and peach-purified lipid-transfer protein (LTP) were prepared as described previously.^{14,15}

Identification of hazelnut allergens

SDS-PAGE immunoblotting. The hazelnut extract and the markers were separated in a discontinuous buffer system in SDS-polyacrylamide 7.5% to 20% separation gradient gel with a 6% stacking gel. The samples were run (0.26 mg/cm per gel) at 6 mA for 16 hours in a BIO-RAD Protein IIX vertical electrophoresis Slab Cell, essentially according to the method of Neville,¹⁶ as already described in detail.¹⁴ Before electrophoresis, the samples were diluted 1:2 in sample buffer containing Tris, 10% SDS, 2-mercaptoethanol, 50% glycerol, and 1% Bromophenol blue and boiled for 5 minutes. After separation, part of the gel was stained with Coomassie Brilliant Blue R-250.

After electrophoresis, the proteins were electroblotted onto either nitrocellulose paper (pore size, 0.45 µm; Amersham, Bucks, United Kingdom) or onto a polyvinylidene difluoride hydrophobic membrane (ProBlott; Applied Biosystems, Foster City, Calif) by using a Trans-Blot Cell from BIO-RAD (Richmond, Calif) at 8°C at 0.4 A for 4 hours. The nitrocellulose blot was blocked, cut into strips, and incubated overnight with patient sera. IgE binding was detected by incubation with iodine 125-labeled anti-human IgE antiserum (Pharmacia & Upjohn) diluted 1:5 in blocking solution and exposure on x-ray film (Amersham Hyperfilm; Amersham, Buckinghamshire, United Kingdom) at -70°C for 4 days.

Study of cross-reactivity

Immunoblotting inhibition experiments were conducted to identify cross-reactivity between hazelnut and birch pollen. Moreover, because the 7 anaphylactic patients reacted to a 9-kD allergen, the hazelnut immunoblotting was also inhibited by peach extract and peach-purified LTP to detect any homology between these 9-kD allergens.

Immunoblotting inhibition experiments. Hazelnut immunoblotting inhibition was carried out with 2 different serum pools (A and B) incubated with serial dilutions of birch extract. Pool A was formed by sera of some of the patients with positive DBPCFC results (nos. 44, 46, 52, 56-59, 62, and 64) and pool B from sera of patients with anaphylactic reactions (nos. 66-72). Briefly, 500 µL of each serum pool at a final dilution of 1:4 was inhibited with 500 µL of different amounts of birch pollen extract at 1, 0.1, 0.01, and 0.001 mg protein content. Immunoblotting inhibition with peach extract and purified peach LTP was performed with a third serum pool (pool C) formed by the sera of the 3 patients with severe anaphylactic reactions who reacted only to the 9-kD allergen (nos. 66, 68, and 72). Pool C was preincubated with either 1, 0.05, and 0.005 mg of total peach extract or 50, 5, and 0.5 µg of purified peach LTP. After 1 hour's incubation, hazelnut extract-blotted strips were incubated overnight with the inhibited sera, following the steps described above.

EAST inhibition experiments with raw and roasted hazelnut. Dose-related EAST inhibition studies were done with the filter-paper disks described and coated with extract of raw or roasted hazelnut. Two serum pools (50 µL) were preincubated with 50 µL of extracts from raw and roasted hazelnut, birch pollen extract, and milk extract as a control applied at the concentrations given in the abscissa of Fig 5. The results were expressed as the percentage inhibition of the maximum signal. One serum pool was formed by sera of patients only reacting to the 18-kD allergen (nos. 47, 48, 53, and 54) and applied in a final dilution of 1:5. Another serum pool (final dilution 1:6) contained sera with IgE against only the 9-kD allergen (nos. 66, 68, 70, 71, and 72).

Purification of the 9-kd allergen by means of HPLC

Purification was achieved by using a 4-step procedure. The first and third steps were done on a High Load Superdex 75 16/60 column (Amersham Pharmacia Biotech, Uppsala, Sweden) equilibrated and eluted with a 35 mmol/L sodium phosphate buffer (pH 7.5) and 0.3 mol/L NaCl. The second step was done on a Cationic Exchange Resource S 1-mL column, with a salt gradient from 0 to 1 mol/L NaCl in 25 column volumes, and the last step was done on a Reverse Phase C 5 column.

Partial N-terminal amino acid sequencing

The electroblotted polyvinyl difluoride membranes were analyzed on an Applied Biosystems 492 Procise sequencer in pulse-liquid mode (Applied Biosystems) to determine the N-terminal partial sequences of the allergenic proteins. All chemicals were from Applied Biosystems.

RESULTS

Determination of allergen-specific IgE antibodies

The clinical characteristics of the patients under investigation have already been published.⁹ Table I gives the CAP-RAST values for hazelnut, Bet v 1, and Bet v 2 for the 65 patients. Table II reports the clinical characteristics and laboratory results of the 7 patients with severe anaphylaxis to hazelnut.

Identification of hazelnut allergens

IgE immunoblotting. All but 2 (nos. 28 and 55) of the 65 patients reacted to an 18-kd band, and other allergens of 32, 35, and 47 kd in molecular weight were recognized as major allergens (Fig 1). Table III shows the different frequency of positive IgE responses to each allergen in the 3 centers.

IgE immunoblotting results of the 7 patients with systemic reactions to hazelnut (Fig 2) showed that they all reacted to the 9-kd protein. IgE binding to the 18- and 14-kd allergens is evident in patient no. 70 with birch allergy. A 14-kd allergen was also recognized by patient no. 69, who, however, did not have birch pollinosis.

Study of cross-reactivity

Immunoblotting inhibition. Fig 3 depicts the IgE immunoblotting of the serum pool A for birch pollen extract, native hazelnut extract, and the inhibition of hazelnut immunoblotting by birch pollen at different concentrations. The IgE binding to the 18-kd major allergen of hazelnut was completely inhibited by 1 mg of birch pollen extract; the binding to only one band of the 14-kd allergen was inhibited by 1 and 0.1 mg of birch extract. IgE reactivity to the allergens at higher molecular weights was completely inhibited by any birch extract concentration. These results demonstrate the high level of homology between birch and hazelnut allergens. Hazelnut IgE immunoblotting with serum pool B, preincubated with birch pollen extract at different concentrations, showed inhibition of the IgE binding to the 14- and 18-kd allergens but not to the 9-kd band (data not shown).

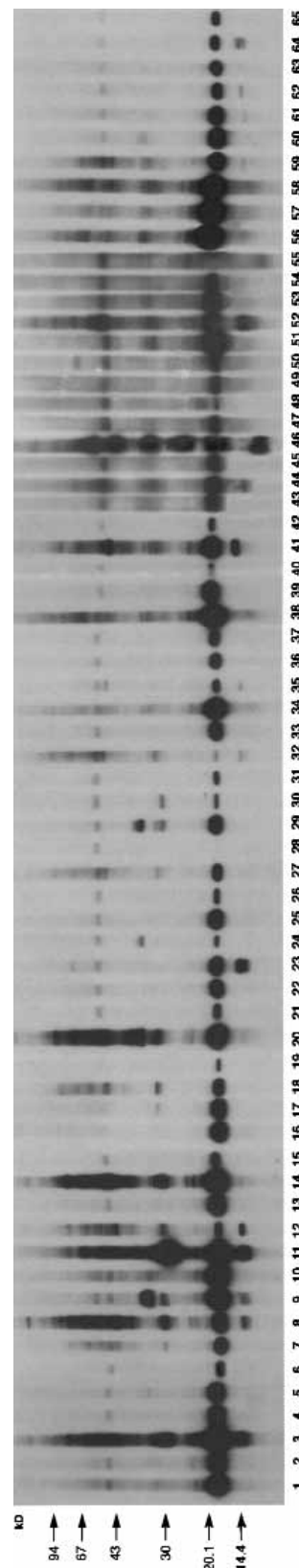


FIG 1. IgE immunoblotting of the native hazelnut extract in sera from 65 patients with positive DBPCFC results.

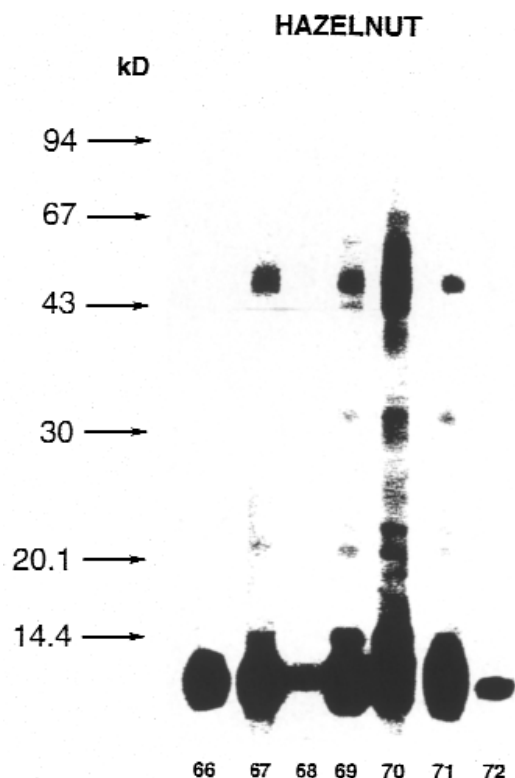


FIG 2. IgE immunoblotting of the native hazelnut extract in sera from 7 patients (nos. 66-72) with systemic reactions on eating hazelnut.

Preincubation of serum pool C with a peach extract caused complete inhibition of IgE binding to the 9-kD band (Fig 4). Peach inhibitor at 0.005 mg did not completely inhibit the binding to hazelnut immunoblotting, whereas 5 μ g of purified LTP did; this is due to the fact that the peach extract contains other proteins beside LTP, and thus it has a minor inhibiting power. The homology of this protein with peach LTP was confirmed by means of the complete inhibition of IgE binding to the 9-kD band by 5 μ g of peach-purified LTP, as shown in Fig 4.

EAST inhibition. EAST inhibition was done with raw and roasted hazelnuts to investigate the heat stability of the hazelnut allergens, preincubating 2 different pools of sera with raw and roasted hazelnut and with birch pollen extract.

Serum pool 9 (patients reacting only to the 9-kD allergen) showed high IgE binding to both raw (EAST class 4) and roasted (EAST class 4) hazelnut. Inhibition of binding to native hazelnut (Fig 5, A) showed that both inhibitor extracts from raw and roasted hazelnut above 10 μ g/mL inhibited IgE binding to the raw nut, thus showing that the allergen to which these sera react is heat resistant. Preincubation with birch pollen extract caused no inhibition. The same result was obtained with extract of roasted hazelnut as the solid phase (Fig 5, B). These findings indicate that the 9 kD-allergen is a heat-stable component in hazelnut, further suggesting homology with peach LTP, which is highly heat stable.

Serum pool 18 (patients responsive only to the 18-kD allergen) showed an EAST value of class 2 toward the

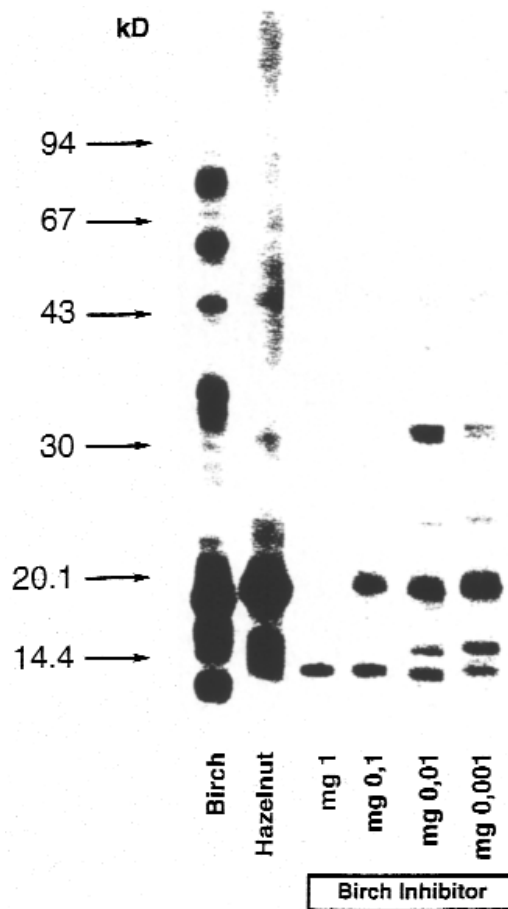


FIG 3. Immunoblotting inhibition of the native hazelnut extract by birch pollen extract with serum pool A from 9 patients with positive DBPCFC results (nos. 44, 46, 52, 56-59, 62, and 64).

raw hazelnut disks and a class 0 result for roasted hazelnut. The inhibition curves showed that IgE binding to raw hazelnut extract was not inhibited by extract from the roasted hazelnut, whereas strong inhibition was obtained with raw hazelnut or birch pollen extract. These results thus indicate that the major allergen of hazelnut is not present in the roasted fruit (Fig 5, C).

Partial N-terminal amino acid sequencing

The N-terminal sequence of the 32-kD band was Val-X-Leu-Gly-Arg-Arg-Ala-Glu-Glu-Gly-X-Asn-Glu-Asp. A search in the SWALL data bank showed it was a member of the 2S albumin family, with 50% sequence identity (similarity, 71.4%) and with a napin-type 2S albumin 1 precursor from soybean (accession no. Q9ZNZ4).

The N-terminal sequence of the 35-kD molecule was Ile-Asp-Val-Gly-Leu-Arg-Arg-Gln-Gln-Gln-Arg-His-Phe-Gly-Glu. A search in the SWALL data base identified this protein as a homologue of a protein belonging to the legumin family with 46.6% sequence identity and 66.6% similarity (legumin fragment from *Calocedrus decurrens*, a species of conifer; accession no. Q39484).

The N-terminal sequence of the 47-kd protein was Ser-X-Glu-GluSer-Tyr-Gly-Glu-Glu-Gln-Asp-X-Asn-Pro-Tyr. A search in the Swiss Prot data bank identified this protein as having a high degree of amino acid sequence identity (53.3%, similarity 73%) with a soybean sucrose-binding protein (accession no. AAF05723).

The N-terminal sequence of the 9-kd purified allergen was Ala-Ile-Ser/Thr-Cys-Gly/Pro-Ser/Gln-Val/Ile-Thr/Lys-Gly-Asn-Leu-Val/Thr/Ala, thus corresponding to 3 isoforms of LTP having various degrees of homology with the peach LTP (accession no. Q9ATH2).

DISCUSSION

In this study we characterized the main allergens of hazelnut on the basis of the IgE reactivity to hazelnut of subjects whose clinical reactions were confirmed by means of DBPCFC.

The major allergen was an 18-kd protein homologous to Bet v 1, as already shown by Hirschwehr et al,¹⁰ who were the first to demonstrate the immunologic relationship between these 2 allergens. The major allergen of birch pollen (Bet v 1) and that of hazelnut (Cor a 1.01) share a high degree of similarity at the amino acid level¹⁷ and show significant IgE cross-reactivity.¹⁸ Patients allergic to birch and hazelnut pollens are often also allergic to eating hazelnut, and almost all patients with hazelnut allergy are allergic to birch and hazelnut pollen. Vieths et al¹⁹ found that the major allergen from the nut had, respectively, a 63% and a 83% amino acid sequence identity with pollen Cor a 1 (X70999) and with the inducible Bet v 1 Sc3 (X77601), thus giving a clear molecular basis to these clinical observations.

The similarity also concerns other proteins common to birch and hazelnut pollens, such as profilin-Bet v 2, which is also present in hazelnut in a homologous form. Thus all the patients with a positive CAP-RAST result for Bet v 1 reacted to the 18-kd band in hazelnut immunoblotting, and those with a positive CAP-RAST result for Bet v 2 reacted to the 14-kd allergen of hazelnut. The complete inhibition of IgE binding to these bands with birch as the inhibitor and using sera of patients allergic to both hazelnut and birch provided evidence that the 18-kd protein of hazelnut we had identified as a major allergen was homologous to Bet v 1. The fact that preincubation of pool A with extract from roasted hazelnut did not inhibit the IgE binding to the extract from raw nuts indicated that roasting destroys this protein, offering a preventive approach on the basis of the use of roasted hazelnut in the production of commercial items. DBPCFCs with roasted hazelnut in comparison with those with the raw material will be needed to confirm the reliability of this solution.

Other proteins were major allergens of hazelnut in our study. First, a 47-kd band toward which all the patients reacted was identified as homologous to a sucrose-binding protein of soybean that mediates the linear uptake of sucrose. This molecule belongs to the vicilin superfamily. As recently observed by one of the authors,¹³ the 47-kd allergen is similar to the P54 vicilin protein of *Pisum*

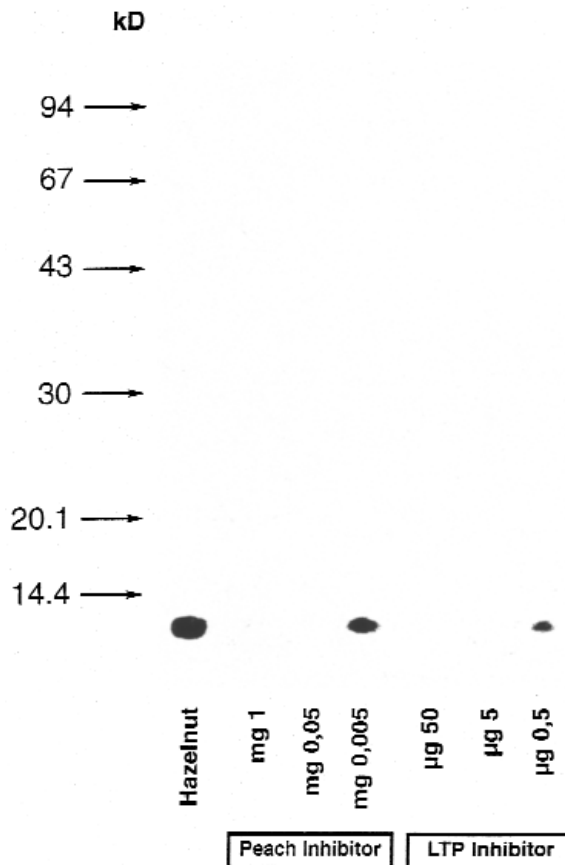


FIG 4. Immunoblotting inhibition of the native hazelnut extract by row: peach extract and peach purified LTP with serum pool C from patients no. 66, 68, and 72.

sativum, which, in turn, is 60% identical to the soybean sucrose-binding protein.²⁰ Sensitization to the 47-kd protein should also be the cause of cross-reactivity with birch higher molecular weight bands because they have recently been shown to contain CCD epitopes that could also be present in hazelnut. Several important food allergens are among the vicilin storage proteins, including Ara h 1 (peanut) and Jug r 2 (walnut), and therefore their cross-reactivity should be evaluated in the future.

Another interesting finding was that a 35-kd legumin was a hazelnut major allergen. Legumins are seed storage proteins belonging to the 11 S globulin family, which includes a 21-kd major allergen of soybean²¹ and a 35-kd protein considered allergenic in coconut.²²

A 32-kd protein was also a major allergen of hazelnut, particularly in the Swiss and Italian patients. It was identified as a component of the 2S albumin family and is significantly homologous to the napin-like 2S albumin precursor of soybean, in which, however, no allergenic 2S albumins are known as yet.

These 3 new allergens we found in hazelnut belong to the group of seed storage proteins, a conspicuous source of plant proteins that are present in the majority of seeds

TABLE I. Summary of data of patients with hazelnut allergy with a positive DBPCFC result

Patient No.	CAP hazelnut	CAP birch		Patient No.	CAP hazelnut	CAP birch		Patient No.	CAP hazelnut	CAP birch	
		Bet v 1	Bet v 2 (profilin)			Bet v 1	Bet v 2 (profilin)			Bet v 1	Bet v 2 (profilin)
1	3	4	0	20	2	5	0	43	2	3	0
2	3	4	0	21	0	3	0	44	3	5	0
3	3	6	3	22	3	4	0	45	2	3	0
4	2	3	0	23	2	5	3	46	3	5	2
5	2	6	0	24	0	3	0	47	3	4	0
6	1	4	0	25	2	3	0	48	2	4	0
7	2	4	0	26	0	3	0	48	3	4	0
8	2	4	2	27	2	4	0	50	2	4	0
9	3	5	2	28	0	3	0	51	3	4	0
10	3	5	0	29	2	4	0	52	2	4	4
11	3	6	3	30	0	3	0	53	2		
12	1	4	0	31	0	3	0	54	2	4	0
13	2	4	0	32	0	2	1	55	4	0	0
14	3	6	0	33	2	4	0	56	3	6	0
15	1	2	0	34	2	6	0	57	3	6	0
16	3	5	2	35	0	3	0	58	3	6	0
17	3	4	0	36	1	4	0	59	2	5	0
18	2	4	0	37	1	5	0	60	2	4	0
19	0	3	0	38	3	6	0	61	2	5	0
				39	0	4	0	62	2	3	0
				40	0	3	0	63	2	4	0
				41	3	6	0	64	2	3	2
				42	0	3	0	65	1	3	0

Results are expressed in CAP classes corresponding to class 1 (0.35-0.7 kU/L), class 2 (0.7-3.5 kU/L), class 3 (3.5-17.5 kU/L), class 4 (17.5-50 kU/L), class 5 (50-100 kU/L), and class 6 (>100 kU/L). Nos. 1-19, Zurich patients; nos. 20-42, Copenhagen patients; nos. 43-65, Milan patients.

TABLE II. Characteristics of patients with hazelnut allergy with systemic reactions

Patient No.	Sex	Age (y)	Symptoms for hazelnut	CAP hazelnut	CAP birch		Symptoms for pollens	Symptoms for foods
					Bet v 1	Bet v 2		
66	F	30	EFIA	2	0	0		Pe, Ap, Pr, Ma, Nu, Pa, Ga, Ce
67	F	20	OAS grade 3	5	0	0	Gr, Pa	Pn, Wa
68	F	19	Anaphylaxis	2	3	0	Gr	Fi, Pe, To, Nu, Ap, Me, Ce, On, Ba, Ry, Oa, Co, So
69	M	24	Anaphylaxis	3	—	—	Gr, Mu	Wa, Pn, Ce, Fe, Ca
70	F	30	Anaphylaxis	3	3	4	Bi, Gr	Pn, Al, Wa
71	F	28	OAS grade 3	3	0	5	Gr	
72	M	34	EFIA	3	—	—		Ma

Results are expressed in CAP classes corresponding to class 1 (0.35-0.7 kU/L), class 2 (0.7-3.5 kU/L), class 3 (3.5-17.5 kU/L), class 4 (17.5-50 kU/L), class 5 (50-100 kU/L), and class 6 (>100 kU/L).

OAS grade 3, Oral allergy syndrome with laryngeal edema; EFIA, exercise food-induced anaphylaxis.

Pollens: Bi, Birch; Gr, grass; Mu, mugwort; Pa, *Parietaria* species.

Foods: Al, Almond; Ap, apple; Ba, barley; Ca, carrot; Ce, celery; Co, corn; Fe, fennel; Fi, fish; Ga, garlic; Ma, maize; Me, melon; Nu, nuts; Oa, oats; On, onion; Pa, pea; Pe, peach; Pr, pear; Pn, peanut; Ry, rye; So, soybean; To, tomato; Wa, walnut.

with very high structural similarities, thus representing hypothetical cross-reacting allergens. However, an elegant study by Bernhisel-Broadbent et al²³ conducted with DBPCFCs in patients allergic to legumes demonstrated that despite the high rate of in vitro cross-reactivity among different legumes, only one or two were responsible for clinical reactions in any single patient. Thus before deciding whether a widespread allergen is

likely to cause cross-reactions, a DBPCFC should always be conducted to verify its clinical role.

Our study also highlighted an IgE response toward a 9-kD LTP specifically occurring in the patients with severe anaphylactic reactions to hazelnut. This allergen was highly cross-reactive with the peach LTP, being similarly heat stable.²⁴

In conclusion, this study confirmed that the most

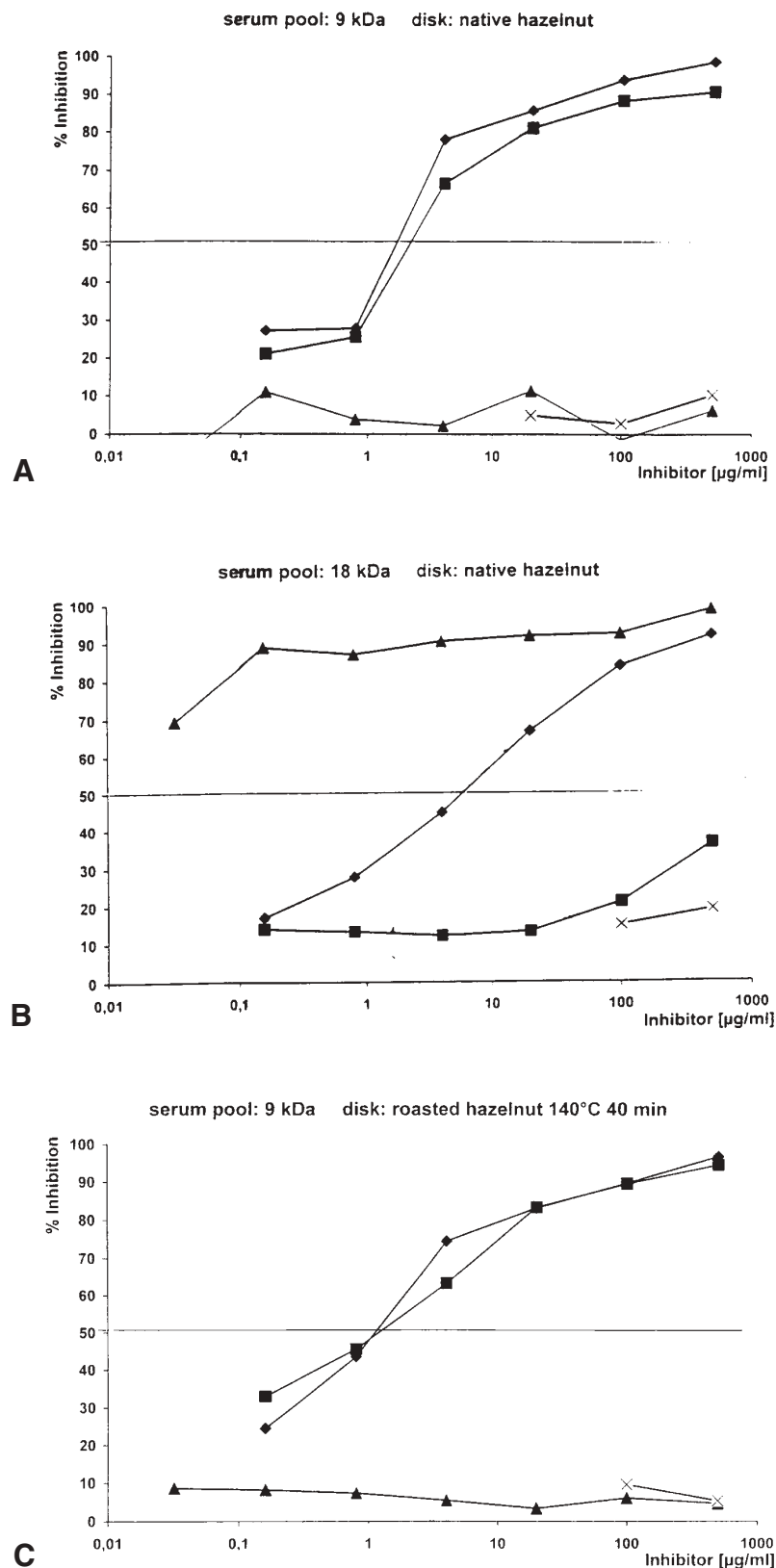


FIG 5. Dose-related EAST inhibition with native (A) and roasted (B) hazelnut with a serum pool containing IgE against a 9-kd protein and with native hazelnut (C) with a serum pool containing IgE against 18-kd protein. ♦, Native hazelnut extract; ■, roasted hazelnut extract; ▲, birch pollen extract; X, milk extract.

TABLE III. Frequency of positive IgE response in patients with a positive DBPCFC result to hazelnut (nos. 1-65) to each allergen in total and in the 3 study centers of Zurich, Copenhagen, and Milan

Molecular weight (kd)	Total (%)	Zurich (%)	Copenhagen (%)	Milan (%)
86	13.8	26.3	—	17.3
84	24.6	36.8	21.7	17.3
66	33.8	42.1	26.0	34.7
60	18.4	47.3	—	13.0
47	95.3	84.2	100	100
44	24.6	31.5	26.0	17.3
35	50.7	52.6	26.0	73.9
32	55.3	57.9	30.4	78.2
30	23.0	36.8	17.3	17.3
24.5	23.0	31.5	13.0	26.0
23.5	29.2	47.3	17.3	26.0
18	96.9	100	95.6	95.6
14.4	24.6	26.3	26.0	21.7
9	3.0	—	—	8.6

important allergen of hazelnut is the 18-kd protein homologous to Bet v 1. It showed 3 new major allergens as proteins of 32, 35, and 47 kd and identified a 9-kd LTP as an allergen causing anaphylactic reactions in Italian patients.

REFERENCES

- Pastorello EA, Rivolta F, Bianchi M, Mauro M, Pravettoni V. Incidence of anaphylaxis in the emergency department of a general hospital in Milan. *J Chromatogr B Biomed Sci Appl* 2001;756:11-7.
- Pumphrey RSH, Stanworth SJ. The clinical spectrum of anaphylaxis in north-west England. *Clin Exp Allergy* 1996;26:1364-70.
- Ewan PW. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996;312:1074-8.
- Stewart AG, Ewan PW. The incidence, aetiology and management of anaphylaxis presenting to an Accident and Emergency department. *Q J Med* 1996;89:859-64.
- Yocum MW, Khan DA. Assessment of patients who had experienced anaphylaxis: a 3-year survey. *Mayo Clin Proc* 1994;69:16-23.
- Sicherer SH, Muñoz-Furlong A, Burks W, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J Allergy Clin Immunol* 1999;103:559-62.
- Sampson HA. Comparative study of commercial food antigen extracts for diagnosis of food hypersensitivity. *J Allergy Clin Immunol* 1998;82:718-26.
- Ortolani C, Ispano M, Pastorello EA, Ansaloni R, Magri A. Comparison of results of skin-prick tests (with fresh foods and commercial extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989;83:683-90.
- Ortolani C, Ballmer-Weber BK, Skamstrup Hansen K, Ispano M, Wüthrich B, Bindslev-Jensen C. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter, study. *J Allergy Clin Immunol* 2000;105:577-81.
- Hirschwehr R, Valenta R, Ebner C, Ferreira F, Sperr WR, Valent P, et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992;90:27-36.
- Caballero T, Pascual C, Garcia-Ara MC, Ojeda JA, Martin-Esteban M. IgE crossreactivity between mugwort pollen (*Artemisia vulgaris*) and hazelnut (*Abellana nux*) in sera from patients with sensitivity to both extracts. *Clin Exp Allergy* 1997;27:1203-11.
- Lowry OH, Rosebrough B, Fair AL, Randall RJ. Protein measurement with the folin phenol reagent *J Biol Chem* 1951;193:265-7.
- Müller U, Lüttkopf D, Hoffman A, Petersen A, Becker W-M, Schocker F, et al. Allergens in native and roasted hazelnuts (*Corylus avellana*) and their cross-reactivity to pollen. *Eur Food Sci Technol* 2000;212:2-12.
- Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borga A, et al. Allergenic cross-reactivity between peach, apricot, plum and cherry in patients with oral allergy syndrome: an in-vivo and in-vitro study. *J Allergy Clin Immunol* 1994;53(suppl 46):55-7.
- Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;103:520-6.
- Neville DM. Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J Biol Chem* 1971;246:6328-34.
- Ipsen H, Hansen OC. The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. *Mol Immunol* 1991;28:1279-88.
- Rohac M, Birkner T, Reimtz I, Bohle B, Steiner R, Breitenbach M, et al. The immunological relationship of epitopes on major tree pollen allergens. *Mol Immunol* 1991;28:897-906.
- Vieths S, Karamloo F, Luettkopf D, Scheurer S, Foetisch K, May S, et al. Recombinant allergens expressed in *E.coli*: benefits and drawbacks in the diagnosis of food allergies. *Arb Paul Ehrlich Inst* 2000;93:159-68.
- Castillo J, Rodrigo MI, Marquez JA, Zuniga A, Franco L. A pea nuclear protein that is induced by dehydration belongs to the vicilin superfamily. *Eur J Biochem* 2000;267:2156-65.
- Helm RM, Cockrell G, Connaughton C, Sampson HA, Bannon GA, Beilison V, et al. A soybean G2 glycinin allergen. I. Identification and characterization. *Int Arch Allergy Immunol* 2000;123:205-12.
- Teuber SS, Peterson WR. Systemic allergic reaction to coconut (*Cocos nucifera*) in two subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: new coconut and walnut food allergens. *J Allergy Clin Immunol* 1999;103:1180-5.
- Bernhisel-Broadbent J, Taylor S, Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates. *J Allergy Clin Immunol* 1989;84:701-9.
- Brenna O, Pompei C, Ortolani C, Pravettoni V, Farioli L, Pastorello EA. Technological process to decrease the allergenicity of peach juice and nectar. *J Agric Food Chem* 2000;48:493-7.