

Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy

Stephan Scheurer, PhD,^a Elide A. Pastorello, MD,^b Andrea Wangorsch, BSc,^a Marion Kästner,^a Dieter Haustein, PhD,^a and Stefan Vieths, PhD^a Langen, Germany, and Milan, Italy

Background: In central and northern Europe food allergy to fruits of the Rosaceae family is strongly associated with birch pollinosis because of the existence of IgE cross-reactive homologous allergens in birch pollen and food. By contrast, in the Mediterranean population allergic reactions to these fruits frequently are not related to birch pollen allergy and are predominantly elicited by lipid transfer proteins (LTPs).

Objective: We sought to determine the prevalence of IgE sensitization to the recombinant cherry allergens Pru av 1 and Pru av 4 in comparison with cherry extract within a representative group of patients who were allergic to cherries recruited in Germany and to compare the relevance of IgE to cherry LTPs in Italian patients.

Methods: Recombinant Pru av 1 and rPru av 4 were available from earlier studies. The cDNA of the cherry LTPs was obtained by using a PCR-cloning strategy. The protein was expressed in *Escherichia coli* and purified by means of metal chelate affinity chromatography. Sera from 101 German patients with birch pollinosis and oral allergy syndrome to cherry and sera from 7 Italian patients with cherry allergy were investigated by using enzyme allergosorbent tests for IgE reactivity with cherry extract, rPru av 1, rPru av 4, and the recombinant cherry LTP. Inhibition experiments were performed to compare the IgE reactivity of natural and recombinant cherry LTPs and to investigate potential cross-reactivity with birch pollen allergens.

Results: The LTP from cherry comprises 91 amino acids and a 26 amino acid signal peptide. The mature cherry LTP shows high amino acid sequence identity with allergenic LTPs from peach (Pru p 3, 88%), apricot (Pru ar 3, 86%), and maize (Zea m 14, 59%) and displays no IgE cross-reactivity with birch pollen. The IgE prevalences in the German patients were as follows: LTP, 3 of 101 (3%); rPru av 1, 97 of 101 (96.0%); rPru av 4, 16 of 101 (16.2%); and cherry extract, 98 of 101 (97%). All 7 Italian patients had IgE against the cherry LTP. **Conclusions:** Recombinant allergens are useful tools for a more accurate in vitro IgE-based diagnosis of cherry allergy. Taken together, they mimic the allergenic activity of cherry extract, having slightly higher biologic activity. Sensitization to the cherry LTP is relevant for a minority of patients recruited

in Germany, but our data indicate that it may be a major allergen in Italy. (J Allergy Clin Immunol 2001;107:724-31.)

Key words: Food allergy, cherry, lipid transfer protein, Pru av 1, Pru av 4, birch pollinosis, cross-reactivity

Birch pollen-associated food allergy is a well-known clinical phenomenon in central and northern Europe. A high percentage of patients allergic to birch pollen have adverse reactions to foods of the Rosaceae family, such as cherry; peach and apricot from the Prunoideae subfamily; and pear and apple, which belong to the Pomoideae subfamily. Patients with allergic reactions to Rosaceae foods have oral allergy syndrome (OAS) and, less frequently, experience systemic reactions.¹⁻³ The mechanisms underlying the development of allergy to Rosaceae foods are not fully clear. Allergic reactions to these foods are due to a primary sensitization to pollen allergens and a subsequent IgE cross-reaction with homologous molecules in food.⁴ Bet v 1,⁵ the major birch pollen allergen, shares common epitopes with the major food allergens (eg, Pru av 1 from cherry,^{6,7} Mal d 1 from apple,⁸ and Pyr c 1 from pear⁹). Moreover, profilins were described as cross-reactive plant panallergens.¹⁰⁻¹² Recently, isoflavone reductase homologous molecules from birch (Bet v 6)¹³ and pear (Pyr c 5)¹⁴ were identified as cross-reactive allergens but were not detected in cherry. Symptoms elicited by pollen-related fruit allergens are usually restricted to the oropharyngeal system, most likely because of the lability of the allergens to digestion in the gastrointestinal tract.

By contrast, in the Mediterranean area Rosaceae food allergy frequently occurs independently of an associated birch pollinosis. Patients allergic to both fruits and birch pollen and patients without pollinosis are sensitized to small, cross-reactive, 9-kD lipid transfer proteins (LTPs).¹⁵⁻¹⁸ Almost all patients with fruit allergy but without pollen allergy are monosensitized to the corresponding LTP. The LTPs Pru p 3^{19,20} from peach and Pru ar 3 from apricot²¹ were identified as major allergens. Mal d 3 from apple²² was found to be a minor allergen in the overall Italian population with apple allergy. LTPs are a class of conserved plant defense-related proteins,²³ and because of their high resistance to pepsin digestion, they are potentially severe food allergens.²⁴

In our previous investigations we have used cherry allergy as a model of pollen-related food allergy.^{6,7,10} The present study was undertaken to investigate the sensitization profile to individual cherry allergens within a

From ^athe Department of Allergology, Paul-Ehrlich-Institut, Langen; and ^bthe Allergy Center, Third Division of General Medicine, Ospedale Maggiore Istituto di Ricerca e Carattere Scientifico, Milan.

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Reprint requests: Stephan Scheurer, PhD, Paul-Ehrlich-Institut, Department of Allergology, Paul-Ehrlich-Str. 51-59, D-63225 Langen, Germany.

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Abbreviations used

EAST: Enzyme allergosorbent test
IUIS: International Union of Immunological Societies
LTP: Lipid transfer protein
OAS: Oral allergy syndrome
5'RACE: Rapid amplification of 5' cDNA ends

representative group of patients with cherry allergy recruited in central and northern Europe and to study the applicability of recombinant allergens for this purpose. The IgE-binding frequency to Pru av 1, the major cherry allergen,⁶ and Pru av 4, the cherry profilin,¹⁰ was determined by means of an enzyme allergosorbent test (EAST). To study the prevalence of IgE specific for cherry LTP in the study population, the complete amino acid sequence of LTP from cherry was deduced from the cDNA sequence, and the recombinant cherry LTP was produced in *Escherichia coli* and characterized.

METHODS

Patients' sera

Sera from 101 patients from Germany were selected on the basis of a reported allergic reaction to cherry and by means of EAST analysis with the corresponding allergen extract. The majority of patients (84/101) had relatively mild OAS-like itching; swelling of the lips, tongue, or throat; or both. Approximately 17% of the subjects had experienced systemic reactions after ingestion of cherries, with respiratory symptoms, rhinitis, or both in 11 patients; gastrointestinal symptoms in 4 patients; and urticaria in 1 patient. All of the patients had a history of birch pollinosis and a positive CAP test result or EAST result to birch pollen extract (data not shown). Almost all of the patients reported symptoms after ingestion of other Rosaceae fruits. Apples and peaches were identified most frequently as offending fruits (Table I). Moreover, most of the patients with cherry allergy were allergic to hazelnuts (79/101), followed by walnuts (33/79). Seventy-one percent of the subjects reported clinical symptoms to at least one kind of vegetable, and a subgroup of 59% had adverse reactions to exotic fruits (eg, kiwi and lychee fruit). Seven additional sera were from Italian patients with cherry allergy who had reactions in open cherry food challenges, which were demonstrated by reaching a maximum dose of 60 g of the fresh fruit.

Protein extracts and allergens

Allergen extract from sweet cherry was freshly prepared by means of a low-temperature method, as described elsewhere.²⁵ Peel extract from cherry was prepared according to the method of Björkstén et al²⁶ by using approximately 25 g of peel in 15 mL of PBS, thus obtaining approximately 30 mL of final extract after dialysis, with a protein concentration of 30 µg/mL. Birch pollen extract was obtained from MAST Diagnostica (Reinfeld, Germany). Recombinant cherry allergens Pru av 1 and Pru av 4 were expressed in *E coli* and purified, as previously described.^{6,10}

RNA purification and first-strand cDNA synthesis

Sweet cherries (*Prunus avium*) were ground to a fine powder with liquid nitrogen. Total RNA was extracted from 100 mg of powder by using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from approximately 1 µg of total RNA by using a degenerated 5'-T₁₆NN-3'-primer (Eurogen-

tec, Searing, Belgium) and the Moloney murine leukemia virus reverse transcriptase (First-Strand cDNA synthesis Kit; Pharmacia, Freiburg, Germany), as recommended by the manufacturer. The reaction was performed at 37°C for 60 minutes, followed by heat denaturation at 99°C for 5 minutes.

PCR of an LTP 3' partial sequence

For cDNA amplification, the degenerated oligonucleotide primers LTP1(190-209) 5'-AAY AAC KYK GCA MGG AC-3' and LTP2(307-327) 5'-TGT GGW GTY AAY ATT CCT TAC-3' (ARK, Darmstadt, Germany) were designed according to the known cDNA sequences of different plant LTPs (PRUDU accession No. X96714; DAUCA accession No. M64746; ARATH accession No. M80567) and the amino acid sequence of Prup3 (accession No. P81402). For the reverse direction, the 3' primer 5'-T₁₆NN-3' was used. The first-strand cDNA amplification was performed in a final volume of 100 µL, containing 0.1 µmol of deoxyribonucleoside triphosphates (Pharmacia), 20 pmol of each primer, and 2 µL of first-strand cDNA synthesis product as a template. After denaturation at 94°C for 1 minute, 2.5 U of Taq DNA polymerase (Pharmacia) was added. The sample was subjected to 30 cycles (1 minute of denaturation at 94°C, 1 minute of annealing at 42°C, and 1 minute of extension at 72°C, with a final extension for 5 minutes at 72°C).

Rapid amplification of 5' cDNA ends

For amplification of the unknown 5' end of the cherry LTP, the rapid amplification of 5' cDNA ends (5'RACE) method was used, according to the manufacturer's instructions (5'-RACE System; GIBCO BRL, New York, NY). First-strand cDNA synthesis was primed with an antisense gene-specific primer (LTP3: 5'-CTT CAC GGT GGC GCA GTT GGT G-3') generated on the basis of the identified 3' partial sequence. A homopolymeric dC-tail was ligated to the 3' end of the purified cDNA by means of a terminal deoxynucleotidyltransferase reaction. The dC-tailed cDNA was amplified by means of PCR with a nested second gene-specific primer (LTP4: 5'-CAG TTG GTG GAG GKG CTG ATC-3') and an abridged anchor primer (enclosed in the 5'-RACE system).

Amplification of full-length LTP-cDNA

The obtained sequences of the 2 overlapping partial cDNAs was used to generate the terminal primers NdeLTPCHpET(+) (5'-CGT CAT ATG CTA ACA TGT GGC CAA GTG TCC AGC-3') and XhoLTPCHpET(-) (5'-GCG CTC GAG TTA CTT CAC GGT GGC GCA GTT GGT GGA GGG GC-3'), corresponding to the cDNA of the mature protein. (Restriction sites are underlined, and start and stop codons are printed in italics.) Forward and reverse primers were applied in a PCR with 0.5 µg of total RNA as a template (1 minute of denaturation at 94°C, 1 minute of annealing at 58°C, and 1 minute of extension at 72°C, with a final extension for 5 minutes at 72°C).

Cloning and sequencing of LTP cDNA

The purified LTP cDNAs were ligated into pGEM-T (pGEM-T Vector System I; Promega, Madison, Wis) or pCRII-TOPO (Invitrogen, Groningen, The Netherlands) and transformed in *E coli* Nov-aBlue cells (Novagen, Schwalbach, Germany). Positive clones were selected by using a blue/white screening and PCR screening with the T7 promoter primer 5'-TAA TAC GAC TCA CTA TAG G-3' and the SP6 primer 5'-GAT TTA GGT GAC ACT ATA GA-3'. DNA sequence analyses of the subsequently isolated plasmids were performed with an ABI 377 stretch automatic fluorescent sequencer (Perkin Elmer, Weiterstadt, Germany).

Expression of recombinant cherry LTP

Ligation of the LTP cDNA into the pET-16b expression vector

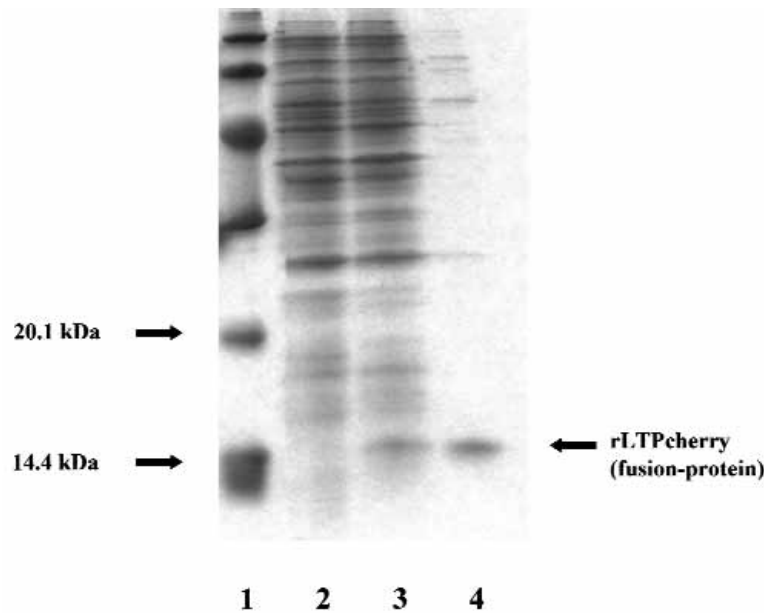


FIG 2. Expression and purification of recombinant LTP (*rLTP*) cherry fusion protein in BL21(DE3) RP codon plus cells. *Lane 1*, Molecular weight marker; *lane 2*, BL21(DE3) RP without isopropyl-D-thiogalactopyranoside, overnight, 37°C; *lane 3*, BL21(DE3) RP codon plus cells with 1 mmol/L isopropyl-D-thiogalactopyranoside, overnight, 37°C; *lane 4*, recombinant LTP cherry fusion protein purified with nickel-nitrilotriacetic acid/agarose.

TABLE I. Case history of German patients with birch pollen allergy with adverse reactions to cherry, other foods, and allergens from nonfood sources

Fruits	Σ = 100	Exotic fruits	Σ = 60	Vegetables	Σ = 72	Nuts	Σ = 83	Other food	Σ = 48	Nonfood source	Σ = 21
Apple	96	Kiwi	52	Carrot	56	Hazelnut	79	Almond	23	Grass pollen	12
Peach	57	Banana	19	Celery	21	Walnut	33	Spice	15	Animal dander	11
Pear	53	Orange	15	Potato	21	Peanut	26	Herb	14	Mite	7
Nectarine	30	Pineapple	10	Tomato	17	Brazil nut	10	Milk	6	Mold	6
Strawberry	25	Mango	3	Paprika	9	Cashew	1	Fish	5	Latex	3
Plum	9	Lychee	3	Soybean	6			Wheat	5	Wasp venom	3
Apricot	8	Lemon	3	Pea	5			Egg	3		
Mandarin	2	Avocado	2	Onion	4			Garlic	2		
Currant	2	Coconut	2	Aubergine	1			Shrimp	1		
Grape	2	Melon	1	Cucumber	1						
Blackberry	1										
Blueberry	1										

detected in *E coli* BL21(DE3) or *E coli* Origami (Novagen) cells (data not shown). The recombinant LTP fusion protein was purified under native conditions by binding of the N-terminal Histag to nickel-nitrilotriacetic acid on the solid phase. Approximately 2 to 3 mg of the purified protein was obtained from 1 L of bacterial culture.

The 3 recombinant allergens Pru av 1, Pru av 4, and cherry LTP are sufficient to detect almost all patients with cherry allergy

One hundred one patients with a clinical history of OAS to cherry were recruited from northern Europe for the determination of the IgE-binding capacity to cherry

extract, rPru av 1, rPru av 4, and recombinant LTP from cherry (Table II). All but 3 subjects showed IgE reactivity to the freshly prepared fruit extract (EAST class 4, *n* = 2; class 3, *n* = 21; class 2, *n* = 59; and class 1, *n* = 16). For 3 patients without IgE reactivity to the extract, EAST classes ranging from 1 to 2 were obtained with the recombinant allergens. Moreover, in an additional 3 subjects with IgE reactivity to the fruit extract, no specific IgE to the recombinant allergens was detectable. Most of the patients (96.0%) were sensitized to the major allergen Pru av 1 (EAST class 4, *n* = 5; class 3, *n* = 22; class 2, *n* = 56; and class 1, *n* = 11). A subgroup of the investigated patient group had IgE specific for cherry profilin (16.2%; EAST class 4, *n* = 0; class 3, *n* = 2; class 2, *n* =

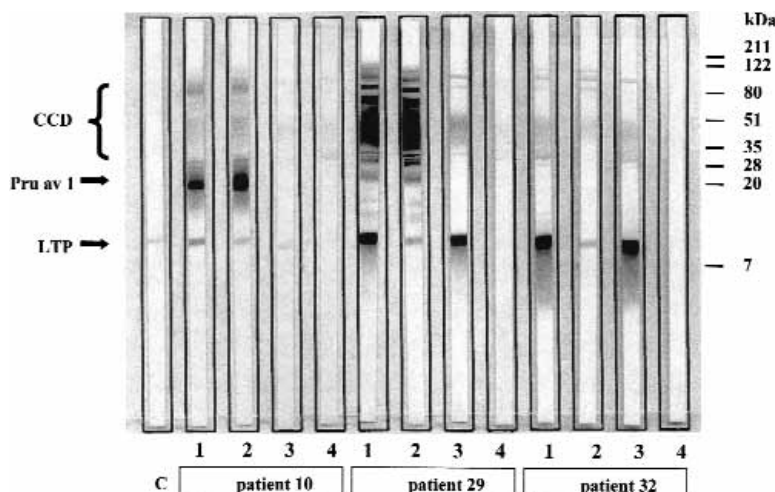


FIG 3. Immunoblot inhibition of patient IgE (sera 10, 29, and 32; 1:10 dilution) to cherry peel extract (20 μ g/cm slot) on the solid phase by means of preincubation with 15 μ g pf cherry recombinant LTP (fusion-protein) (lane 2), 100 μ g of birch pollen extract (lane 3), and 100 μ g of cherry peel extract (lane 4) and without inhibitor (lane 1). Serum from a nonallergic subject (C) was used as control. Immunodetection was performed with a mouse-anti human IgE alkaline phosphatase-labeled mAb (1:1000). CCD, Cross-reactive carbohydrate determinants.

10; and class 1, $n = 4$) and the cherry recombinant LTP (3.0%; EAST class 4, $n = 0$; class 3, $n = 2$; class 2, $n = 1$; and class 1, $n = 0$). Only one patient (patient 32) without sensitization to Pru av 1 showed specific IgE to the cherry LTP and the cherry profilin. The EAST determination was extended to 7 sera from Italian patients with cherry allergy (Table II). All sera were EAST positive for cherry recombinant LTP, and 2 of 7 sera were positive for the birch pollen-related allergens Pru av 1 and Pru av 4, respectively. No IgE binding to the coupled proteins was observed with a serum from a nonallergic control subject. All EAST determinations were performed twice.

LTP-specific IgE without cross-reactivity to birch pollen allergens

Three sera were selected for IgE immunoblot inhibition with cherry peel extract on the solid phase and preincubation with purified recombinant LTP, cherry peel extract, and birch pollen extract as inhibitors (Fig 3). For 2 sera (No. 29 and 32), IgE binding to the natural LTP was strongly inhibited after preincubation with the recombinant cherry LTP and with cherry peel extract. No inhibition of IgE reactivity was obtained after preincubation with birch pollen extract. Pru av 1-specific IgE from patient 10, who was monosensitized to the 18-kd major cherry allergen (Table I), was completely inhibited by cherry and birch extract. Moreover, preincubation of serum 29 with birch pollen extract completely inhibited the IgE reactivity to Pru av 1 (weakly detected on the immunoblot) and to proteins with molecular weights from approximately 30 to 80 kd, possibly carrying cross-reactive carbohydrate determinants.³² A serum from a nonallergic subject showed no IgE reactivity with the blotted proteins.

DISCUSSION

The frequency of IgE sensitization to the recombinant cherry allergens Pru av 1 and Pru av 4 and cherry extract was investigated within a representative group of 101 patients with cherry allergy recruited from northern Europe. All patients had a clear clinical history of birch pollinosis with associated food allergy to cherry. Because LTPs from peach and apricot are known as important allergens in the Mediterranean population, a recombinant cherry LTP was produced in *E coli* and included in the study.

IgE sensitization to the cherry allergens was determined by means of EAST analysis. In comparison with freshly prepared cherry extract, almost all sera were detected as IgE positive by using the 3 recombinant cherry allergens. Ninety-seven (96.0%) of the 101 patients allergic to birch pollen showed IgE reactivity with the major cherry allergen Pru av 1, which is well in accordance with previous results in a smaller group of patients.⁶ Sixteen percent of the patients were sensitized to the cherry profilin, Pru av 4. The fact that in 3 sera IgE specific for cherry extract were not detectable is probably caused by a low relative content of the relevant allergens in the whole protein extract. Negative IgE detection to all 3 recombinant proteins in 3 of 101 patients was probably caused by a sensitization to cross-reactive carbohydrate determinants or Pru av 2.³³ Only 3 sera of the group with cherry and birch pollen allergy showed specific IgE antibodies to cherry LTP (3.0%). By contrast, all sera from Italian patients presented LTP-specific IgE, and sensitization to Pru av 1 appears less common in the Mediterranean. Within the investigated patient group from northern Europe, the cherry LTP was characterized as a minor allergen. Considering the known importance of Pru p 3 and Pru ar 3 as fruit allergens,^{20,21} it is likely that the

TABLE II. Specific IgE to cherry allergens determined by means of EAST analysis in 108 patients with adverse reactions to cherry

Serum	Recombinant				Serum	Recombinant			
	LTP	rPru av 1	rPru av 4	Extract		LTP	rPru av 1	rPru av 4	Extract
1	3	4	0	3	55	0	1	0	1
2	0	3	1	4	56	0	2	0	2
3	0	3	0	3	57	0	3	0	3
4	0	4	0	3	58	0	2	0	3
5	0	3	0	2	59	0	1	1	1
6	0	0	0	1	60	0	2	0	2
7	0	2	0	2	61	0	1	0	0
8	0	2	0	1	62	0	3	0	2
9	0	2	0	2	63	0	2	0	2
10	0	4	0	3	64	0	2	2	2
11	0	2	0	2	65	0	2	0	2
12	0	2	2	2	66	0	2	0	1
13	0	2	0	2	67	0	1	0	2
14	0	2	0	2	68	0	3	3	3
15	0	2	0	1	69	0	0	0	1
16	0	3	0	3	70	0	1	0	1
17	0	2	0	2	71	0	2	0	1
18	0	2	0	2	72	0	2	0	2
19	0	0	0	3	73	0	3	0	3
20	0	3	0	3	74	0	2	1	2
21	0	2	0	2	75	0	3	2	3
22	0	2	0	2	76	0	2	0	2
23	0	3	0	3	77	0	3	2	3
24	0	3	0	3	78	0	1	0	2
25	0	2	0	2	79	0	2	0	2
26	0	2	0	2	80	0	2	0	2
27	0	2	0	0	81	0	2	2	2
28	0	2	0	3	82	0	0	*	2
29	2	2	0	2	83	0	2	0	2
30	0	1	0	2	84	0	2	0	2
31	0	2	0	2	85	0	1	0	2
32	3	0	2	2	86	0	2	0	2
33	0	0	3	2	87	0	2	1	1
34	0	1	0	1	88	0	2	0	2
35	0	2	0	2	89	0	3	2	3
36	0	2	0	0	90	0	3	2	2
37	0	2	0	2	91	0	4	2	4
38	0	1	0	1	92	0	2	0	2
39	0	2	0	2	93	0	3	0	3
40	0	4	0	3	94	0	3	0	3
41	0	3	0	3	95	0	2	*	2
42	0	3	0	2	96	0	2	0	2
43	0	2	0	2	97	0	2	0	2
44	0	3	0	2	98	0	3	1	2
45	0	3	0	2	99	0	2	0	2
46	0	1	0	1	100	0	1	0	1
47	0	2	0	2	101	0	2	0	2
48	0	2	0	1	11	2	0	0	*
49	0	2	0	1	12	1	0	0	2
50	0	2	0	2	13	2	0	0	2
51	0	2	0	2	14	2	1	0	2
52	0	2	2	2	15	2	0	2	2
53	0	2	0	2	16	2	2	2	2
54	0	2	0	2	17	3	0	0	2

Sera 1 to 101 are from German patients, and sera 11 to 17 are from Italian patients. Cherry extract, recombinant LTP from cherry, the major cherry allergen Pru av 1, and the cherry profilin (Pru av 4) were coupled to the solid phase. Among German patients, 3 (3.0%) of 101 had specific IgE to recombinant LTP, 97 (96.0%) of 101 had specific IgE to rPru av 1, 16 (16.2%) of 99 had specific IgE to rPru av 4, and 98 (97.0%) of 101 had specific IgE to cherry extract.

*No serum available.

cherry LTP will represent the major allergen in patients from southern Europe. According to the IUIS allergen nomenclature,³¹ the cherry LTP was submitted to the official IUIS allergen list as Pru av 3.

The identified cherry LTP comprises a length of 117 amino acids, with a typical signal peptide and cleavage site.²⁹ The mature protein is a member of the LTP family of 9-kD proteins, which play a role in the transfer of phospholipids and galactolipids across membranes, possibly in the wax and cutin deposition in the cell walls,³⁴ and in the defense against bacterial and fungal pathogens.²³ The identified cherry LTP showed high amino acid sequence identity with LTPs of the Prunoideae subfamily, Pru p 3 from peach (88%),²⁰ and Pru ar 3 from apricot (86%).²¹ Both allergens were recently described as cross-reactive major allergens responsible for clinical symptoms in patients with food allergy in the Mediterranean area. The fact that LTPs are highly resistant to digestive enzymes²⁴ and that a subgroup of Italian patients without birch pollinosis is monosensitized to LTPs indicates a potential primary sensitization to LTPs through the ingestive route.²¹ In contrast, birch pollen-associated food allergies are the consequence of a primary sensitization by birch allergens and a cross-reaction of IgE antibodies with homologous proteins of the fruits.⁴ The results of inhibition experiments performed in this study underlined that a sensitization to cherry LTP is not related to a previous sensitization to birch pollen allergens. Moreover, the strong inhibition of IgE binding to natural LTP after preincubation of serum with the recombinant protein demonstrated similar immunologic properties of these 2 molecules. To explain the differences in the pattern of reactivity between the northern and southern European allergic population, we have to consider the existence of LTPs in pollen predominantly grown in the Mediterranean area as cross-reactive allergenic sources (eg, ragweed pollen and Par j 1 from *Parietaria judaica*),³⁴ which possibly can be responsible for a potential primary sensitization through the inhalative route.

In conclusion, recombinant cherry allergens are useful tools for a more accurate in vitro diagnosis of the corresponding food allergy. Our data indicate that it will be possible to replace cherry extract with 3 recombinant allergens without affecting the results of in vitro diagnosis and with the benefit of providing information on the sensitization to individual components. The application of recombinant allergens in the in vitro diagnosis is beneficial because allergenic extracts from fruits are known to be labile. The cherry LTP was identified as a new pollen-independent allergen in a minority of patients with birch pollen allergy and OAS after ingestion of cherry. Moreover, the new allergen Pru av 3 will supply new information on the sensitization of patients with cherry allergy with and without birch pollinosis, especially in patients from the Mediterranean area.

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REFERENCES

1. Caballero T, Martin-Esteban M. Association between pollen hypersensitivity and edible vegetable allergy: a review. *J Invest Allergol Clin Immunol* 1998;1:6-16.
2. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollen, with and without symptoms of food allergy. *Clin Exp Allergy* 1994;24:367-74.
3. Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982;37:437-43.
4. Kazemi-Shirazi L, Pauli G, Purohit A, Spitzauer S, Fröschel R, Hoffmann-Sommergruber K, et al. Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J Allergy Clin Immunol* 2000;105:116-25.
5. Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al. The gene coding for the major birch pollen allergen Bet v 1 is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935-8.
6. Scheurer S, Metzner K, Hausteiner D, Vieths S. Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. *Mol Immunol* 1997;34:619-29.
7. Scheurer S, Son DY, Boehm M, Karamloo F, Franke S, Hoffmann A, et al. Cross-reactivity and epitope analysis of Pru a 1, the major cherry allergen. *Mol Immunol* 1999;36:155-67.
8. Son DY, Scheurer S, Hoffmann A, Hausteiner D, Vieths S. Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. *Eur J Nutr* 1999;38:201-15.
9. Karamloo F, Scheurer S, Wangorsch A, May S, Hausteiner D, Vieths S. Pyr c 1, the major allergen (*Pyrus communis*) is a new member of the Bet v 1 allergen family. *J Chromatography B* 2001. In press.
10. Scheurer S, Wangorsch A, Nerkamp J, Skov PS, Ballmer-Weber B, Wüthrich B, et al. Cloning of the panallergen profilin from pear (Pyr c 4), cherry (Pru av 4) and apple and its cross-reactivity with birch pollen profilin Bet v 2. *J Chromatography B* 2001. In press.
11. Valenta R, Duchene M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;253:557-60.
12. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.
13. Karamloo F, Schmitz N, Scheurer S, Foetisch K, Hoffmann A, Hausteiner D, et al. Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. *J Allergy Clin Immunol* 1999;104:991-9.
14. Vieths S, Karamloo F, Lüttkopf D, Scheurer S, Foetisch K, May S, et al. Recombinant allergens expressed in *E. coli*: benefits and drawbacks in the diagnosis of food allergies. In: Kurth R, Hausteiner D, eds. *Regulatory control and standardization of allergenic extracts*. Langen, Germany: Arbeiten aus dem Paul-Ehrlich-Institut; 2000. p. 159-68.
15. Asero R. Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. *Ann Allergy Asthma Immunol* 1999;83:377-83.
16. Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borgia A, et al. Allergic cross-reactivity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: an in vivo and in vitro study. *J Allergy Clin Immunol* 1994;94:699-707.
17. Sanchez-Monge R, Lombardero M, Garcia-Selles FJ, Barber D, Salcedo G. Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999;103:514-9.
18. Pastorello EA, Incorvaia C, Pravettoni V, Farioli L, Conti A, Vigano G, et al. New allergen in fruit and vegetables. *Allergy* 1998;52:48-51.
19. Pastorello EA, Ortolani C, Baroglio C, Pravettoni V, Ispano M, Giuffrida MG, et al. Complete amino acid sequence determination of the major allergen of peach (*Prunus persica*) Pru p 1. *Biol Chem* 1999;380:1315-20.
20. 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.
21. Pastorello EA, D'Ambrosio FP, Pravettoni V, Farioli L, Giuffrida MG, Monza M, et al. Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000;105:371-7.
22. Pastorello EA, Pravettoni V, Farioli L, Ispano M, Fortunato D, Monza M,

- et al. Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;104:1099-106.
23. Gacia-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995;3:72-4.
24. Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier M-F, Ciurana CLF, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000;122:20-32.
25. Vieths S, Schöning B, Petersen A. Characterization of the 18-kDa apple allergen by two-dimensional immunoblotting and microsequencing. *Int Arch Allergy Immunol* 1994;104:399-404.
26. Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980;35:671-7.
27. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
28. Ceska M, Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972;9:1021-30.
29. Nielsen H, Engelbrecht J, Brunak S, von Heijne G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 1997;10:1-6.
30. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* 1988;85:2444-8.
31. King TP, Hofmann D, Loewenstein H, Marsh D, Platt-Mills TAE, Thomas W. Allergen nomenclature. *Int Arch Allergy Immunol* 1994;105:224-33.
32. Fötisch K, Altmann F, Hausteil D, Vieths S. Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int Arch Allergy Immunol* 1999;120:30-42.
33. Inschlag C, Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, Ebner C, Scheiner O, et al. Biochemical characterization of Pru av 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol* 1998;116:22-8.
34. Duro G, Colombo P, Costa MA, Izzo V, Porcasi R, Di Fiore R, et al. Isolation and characterization of two cDNA clones coding for isoforms of the *Parietaria judaica* major allergen Par J 1.0101. *Int Arch Allergy Immunol* 1997;112:348-55.