

Wine Anaphylaxis in a German Patient: IgE-Mediated Allergy against a Lipid Transfer Protein of Grapes

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Key Words

Allergy · Anaphylaxis · Basophil activation test · Grapes · Lipid transfer protein · Wine

Abstract

Background: IgE-mediated allergy to grapes has very rarely been reported in patients from the Mediterranean area. Recently, endochitinase 4 and a lipid transfer protein (LTP) have been identified as major allergens in grape-allergic patients who do not have an associated pollinosis. The purpose of this case study was to identify the allergens responsible for severe anaphylactic reactions after consumption of wine, fresh grapes and raisins in a German patient. **Methods:** Prick-to-prick tests and the basophil activation test (BAT) were performed to confirm allergy. Specific IgE was further analyzed by immunoblotting and inhibition tests for the determination of crossreactivity. The IgE-binding protein was subjected to N-terminal microsequencing. **Results:** Prick-to-prick tests were positive to fresh and cooked white and blue grapes, to raisins, to white and red wine, and to grape extract. Specific IgE against grapes (f259) was 2.43 kU/l (class 2). The BAT showed specific IgE-mediated activation of basophils after stimulation with grape extract. IgE binding to a 15-kDa protein was completely

inhibited by pre-incubation with recombinant cherry LTP Pru av 3. N-terminal sequencing identified this 15-kDa protein as grape LTP Vit v 1. **Conclusion:** Our data show that sensitization to LTP can occur outside the Mediterranean area causing severe fruit allergy without association to pollinosis.

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Introduction

In adults, food allergies in central and northern Europe are usually due to crossreactivity of IgE which is directed to inhalant allergens, e.g. birch pollen. Respiratory symptoms to the sensitizing allergen precede the food allergy but must not always be present. This type of pollen-related food allergy develops mainly to fruits of the *Rosaceae* family, nuts and vegetables. In contrast, in the Mediterranean area, IgE-mediated fruit and vegetable allergies are not associated with pollinosis, and sensitization to these foods appears to take place via the gastrointestinal tract. In these patients, the major allergens have been identified as lipid transfer proteins (LTPs), e.g. in peach, apple, cherry and apricot. LTPs are stable to heat and digestion and show no IgE crossreactivity to pollen allergens [1, 2].

After wine consumption, symptoms like flush, rhinitis, asthma, or migraine are not rare. Such symptoms could be caused by an immediate-type reaction to grapes or molds as well as by intolerance reactions to histamine and sulfite. Grapes belong to the *Vitis vinifera* species of the *Vitaceae* family. Grapes grown in temperate and subtropical areas on all continents are widely consumed as fresh fruit, juice and wine. However, allergy to grapes has been rarely reported [3–8]. In a recent study, endochitinase 4, LTP Vit v 1 (accession No. P80274) and a thaumatin-like protein were identified as grape and wine allergens in Italian patients [9]. Here, we report the symptoms and the results of in vivo and in vitro diagnostic procedures in a patient without atopic disease who had repeated anaphylactic reactions after consumption of wine, fresh grapes and raisins, and the allergen responsible for these reactions was identified as LTP Vit v 1.

Patient and Methods

Patient

Within 2 years, a 27-year-old woman had several episodes with palmoplantar pruritus, angioedema of the lips, eyelids and tongue, dyspnea, dysphagia and tachycardia 20–60 min after drinking red wine and champagne and eating fresh white grapes and raisins. She had no history of atopic disease, previous food allergy (including oral allergy syndrome) or drug hypersensitivity. Her family is not of Mediterranean descent. After completion of the in vitro and in vivo investigations, we advised our patient to avoid all kinds of grapes, raisins, grape juice, wine and champagne. She can consume grape sugar (glucose) vinegar, and any kind of apples and cherries without allergic symptoms.

Skin Tests

Skin prick tests (SPT) with commercially available allergens (pollen from grass, rye, birch, alder, hazel, beech, mugwort, cat and dog danders, house dust mites, egg, milk, herring and hazel nut; ALK-Scherax, Hamburg, Germany) and standardized prick-to-prick tests with white and blue grapes, raisins, grape juice, wine, and vinegar were performed. Grapes were tested raw and cooked in a microwave oven (900 W, 30 s). In the test, + was defined as a positive reaction with a wheal diameter of 2–4 mm, ++ as a positive reaction with a wheal diameter of 4–6 mm and +++ as a positive reaction with a wheal diameter >6 mm. All tests were performed according to the recommendations of the European Academy of Allergy and Clinical Immunology [10].

Determination of Grape-Specific IgE

Serum was analyzed for grape-specific IgE antibodies by the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden) according to the instructions of the manufacturer.

Grape Extract

Protein extract was prepared from fresh white grapes using a low-temperature acetone-powder method as described for apple [11].

Basophil Activation Test

Blood (5 ml) from the patient and a non-allergic control person was used within 6 h of blood sampling for the basophil activation test (BAT). The BAT, also called flow-cytometric cellular allergen stimulation test, is based on the in vitro allergen-induced specific activation of basophils [12]. The assay was performed using a kit according to the instructions of the manufacturer (Flow-CAST®; Bühlmann Laboratories, Basel, Switzerland). Briefly, leukocytes were stimulated in vitro with grape extract at concentrations ranging from 1 to 10^{-10} µg/ml, control antigen (mite mix) and a positive control (activating anti-FcεRI antibody). As there is limited experience with the basophil activation capacity of LTPs, a concentration range between 1 and 10^{-10} µg/ml of grape extract was investigated. The cells were double stained with anti-CD63-PE- and anti-IgE-FITC-labeled antibodies. Activated basophils (CD63+ and IgE+ double-positive cells) were counted by flow cytometry at 488 nm on a FACS Calibur (Becton Dickinson Immunocytometry Systems, Mansfield, Mass., USA) using Cell Quest Software.

Recombinant Cherry LTP

Pru av 3 was expressed as His-tagged protein and purified from *Escherichia coli* as described [13].

SDS-PAGE and Immunoblotting

Grape extract (10 µg protein/cm for Coomassie staining and 70 µg/cm for immunoblotting) was separated under non-reducing conditions by SDS-PAGE in a 17.5% separation gel using a BioRad (Munich, Germany) Mini Protean cell. The gel was either subjected to a total protein stain with Coomassie brilliant blue, or proteins were transferred onto a 0.2-µm nitrocellulose membrane (Schleicher und Schüll, Dassel, Germany) by tank blotting (Hoefer Pharmacia Biotech, San Francisco, Calif., USA) for 45 min at 100 V. Transfer was controlled by reversible staining of membranes with Ponceau S (Sigma Diagnostics, St. Louis, Mo., USA). For the detection of allergen-specific IgE antibodies, serum from the patient and a non-allergic control was diluted 1:5 and incubated with the blot strips overnight. After washing, bound IgE antibodies were detected with alkaline-phosphatase-conjugated monoclonal mouse anti-human IgE (1:750; PharMingen, San Diego, Calif., USA). The binding patterns were visualized using the CSPD chemiluminescent Kit (Applied Biosystems, Bedford, Mass., USA). For N-terminal sequencing, grape extract was separated by Tricine SDS-PAGE according to Schägger and von Jagow (20 µg/cm, non-reducing conditions, T = 16%) [14] and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (BioRad).

Inhibition Assay

To investigate crossreactivity with a recombinant LTP, the patient's serum was pre-incubated with 15 µg Pru av 3, and then added to a blot strip with separated grape extract. The following steps were performed as described above.

N-Terminal Microsequencing

N-terminal amino acid sequencing was performed with a Pro-cise 492 protein sequencer in pulsed liquid mode (Applied Biosystems, Weiterstadt, Germany).

Results

Skin Prick Tests and Specific IgE

SPT with commercially available allergens were positive to mugwort pollen, hazelnut and apple. Standardized prick-to-prick tests revealed positive reactions to fresh and cooked, white and blue grapes, to grape juice, raisins, and white and red wine (table 1, fig. 1). SPTs with different vinegars were negative. Total IgE was 46 kU/l, specific IgE to grape (f259) was 2.43 kU/l (class 2).

Basophil Activation Test

Activation of the patient's basophils after stimulation with grape extract at different concentrations yielded 46 and 37% activated basophils at 1 and 10^{-6} $\mu\text{g/ml}$, respectively, compared to 6.5% background of the negative control (fig. 2). Activation of basophils was still induced (30 and 12%) at very low concentrations of grape extract (10^{-10} and 10^{-12} $\mu\text{g/ml}$, respectively). The results may indicate that grape LTP has high allergenic potency.

In contrast, in grape-extract-stimulated samples of the control person, basophil activation was negative with all antigen concentrations, i.e. 5.7 and 6% activated basophils at 1 and 10^{-6} $\mu\text{g/ml}$, respectively (fig. 2).

Stimulation with control antigen (mite mix) was negative (6 and 4.5%), and positive controls with activating anti-Fc ϵ RI antibody yielded basophil activation (40 and 69%) in both the patient and the control person.

SDS-PAGE and Immunoblot

To visualize the protein pattern of grapes, one gel was stained with Coomassie brilliant blue (fig. 3a). Multiple bands were stained between 21 and ~50 kDa, but not below 20 kDa. To achieve a reliable sensitivity in the immunoblotting assay, and for subsequent microsequencing of a low-molecular-weight band, the amount of total protein was increased from 10 to 20 up to 70 $\mu\text{g/cm}$. Under these conditions, IgE immunoblotting revealed a single strong band with an apparent molecular weight of ~15 kDa, which was completely inhibited by pre-incubation of the serum with 15 μg of purified recombinant Pru av 3, the LTP from cherry (fig. 3b, lane 3). No specific detection was observed with the serum of a non-allergic donor (fig. 3b, lane 1).

Sequence Analysis

To confirm the hypothesis that the 15-kDa band represented an LTP, grape extract (20 $\mu\text{g/cm}$) was separated by tricine gel electrophoresis, transferred to PVDF and stained with Coomassie. The band with an apparent mo-

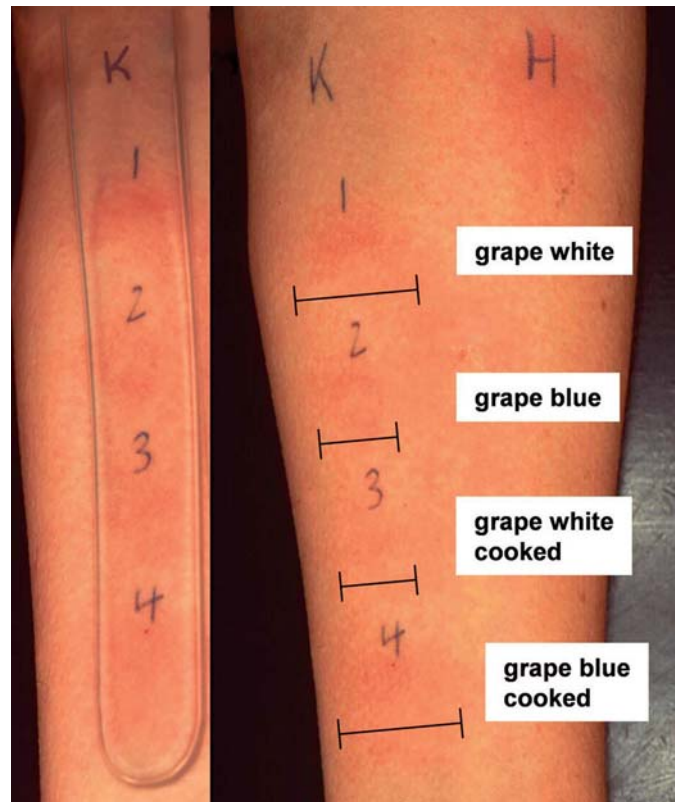


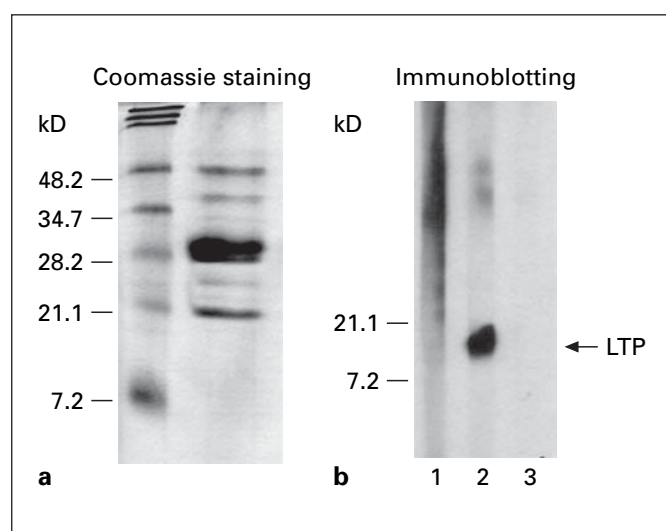
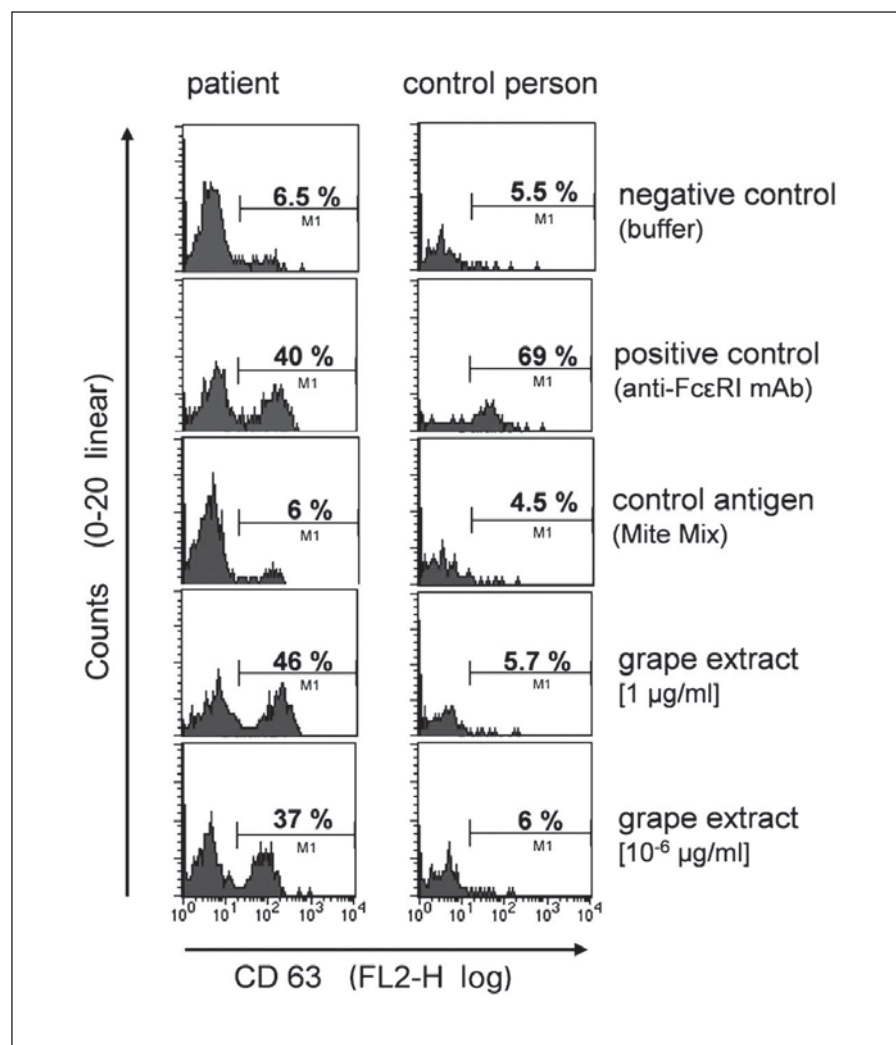
Fig. 1. Examples of prick-to-prick tests with positive reactions to white (1) and blue grapes (2), fresh and cooked in a microwave oven (900 W, 30 s; 3 and 4, respectively). K = Negative control; H = positive control (histamine). Bars indicate wheal diameters.

Table 1. Results of prick-to-prick tests with grapes, wine, raisins, grape juice, grape sugar (glucose) and vinegar

	Raw	Cooked
Grape white	++	++
Grape blue	++	++
White wine	+	ND
Red wine	++	ND
Raisin	+++	ND
Grape juice	++	ND
Grape sugar (glucose)	–	ND
Vinegar	–	ND

Grapes were tested raw and cooked in a microwave oven (900 W, 30 s). ND = Not determined; + = positive reaction with a wheal diameter of 2–4 mm; ++ = positive reaction with a wheal diameter of 4–6 mm; +++ = positive reaction with a wheal diameter >6 mm.

Fig. 2. BAT: blood cells were stimulated in vitro with allergens (grape extract and control antigens), double stained with anti-IgE and anti-CD63 antibodies and flow-cytometrically analyzed. Histograms show gated IgE-positive cells analyzed for the activation marker CD63. At different concentrations, basophil activation after stimulation with grape extract was positive in the patient (46 and 37%) and negative in the control person (5.7 and 6%). BAT with control antigen (mite mix) was negative in both the patient and the control.



lecular weight of 15 kDa was excised and subjected to the Edman degradation, resulting in the amino acid sequence TVT(C)GQVASAL which was identical to the N-terminal sequence of the grape LTP P4 (P80274) and to the amino acid sequence of Vit v 1 identified by Pastorello et al. [9], except that the N-terminal sequence of Vit v 1 was truncated by two residues.

Fig. 3. **a** SDS-PAGE of grape extract (right lane). The left lane represents molecular-weight-marker proteins. The gel was stained with Coomassie brilliant blue. **b** Immunoblotting and inhibition assay. Lane 1 = Non-allergic control; lane 2 = IgE binding of the wine-allergic patient; lane 3 = pre-incubation of patient serum with 15 µg of purified recombinant cherry LTP Pru av 3.

Discussion

In central and northern Europe, patients sensitized to birch pollen frequently develop IgE-mediated allergic reactions to fruits and vegetables. Most patients allergic to birch pollen react to raw apples, hazelnut, peach, apricot, cherry, carrot, celery and numerous other fruits and vegetables. This syndrome is mainly caused by homologues of Bet v 1, the major birch pollen allergen, belonging to the PR-10 family of 'pathogenesis-related proteins' [15]. Most of these allergens are heat labile and, therefore, destroyed by cooking. Relevant examples are Pru av 1 from cherry [16] or Mal d 1 from apple [17].

In the Mediterranean area, *Rosaceae* fruits, particularly peaches, are among the most frequent causes of food-induced allergic symptoms. Most of this area is virtually free of birch trees. Accordingly, the vast majority of the *Rosaceae*-fruit-allergic patients living in the Mediterranean area do not have specific IgE against Bet v 1. Heat-stable LTPs have been identified as major food allergens in the Mediterranean population [18]. Almost all patients with fruit allergy and without pollinosis are monosensitized to LTPs [1]. The LTPs Pru p 3 from peaches [19] and Pru ar 3 from apricots [20] have been identified as major allergens, and Mal d 3 from apples as a minor allergen. Allergenic LTPs have also been detected and characterized in other plant foods, such as chestnuts [21], hazelnuts [22], maize [23], asparagus [24] or lettuce [25].

LTPs are a class of conserved plant proteins widely distributed in nature [26]. They belong to a family of polypeptides (90–95 amino acids) involved in plant defense mechanisms against pathogens and environmental stress [27]. In addition, they are thermostable [28] and extremely resistant to pepsin digestion [29].

In our study, we performed standardized prick-to-prick tests and an in vitro BAT to confirm that anaphylactic reactions after consumption of wine, fresh grapes and raisins were due to an IgE-mediated allergy. However, the IgE-binding band appeared at approximately 15 kDa, which does not correspond to the expected molecular weight of an LTP. This result may be due to an imprecise molecular weight determination in the Laemmli system of small proteins. This is in accordance with an earlier report by Pastorello et al. [30], who observed a comparable molecular weight for peach LTP. However, since in our study N-terminal sequencing and inhibition data confirmed that the 15-kDa allergen represented an LTP [31], there is no doubt on the identity of the allergen.

In the immunoblotting experiment (fig. 3), two weak bands were detected at approximately 35 and 50 kDa, which appeared to be partially inhibited by rPru av 3. This phenomenon may be due to low-level oligomerization of the grape LTP caused by the sample treatment prior to SDS-PAGE.

Although the grape-allergic patient showed cross-reactive IgE to Pru av 3, the LTP from cherry, cherries were tolerated. The inhibition experiment confirmed that the low-molecular-weight band represented an LTP, but also indicated a clinically insignificant cross-reaction. The presence of clinically irrelevant IgE is a well-known phenomenon in both pollen-related and classical food allergy. Recently, we found a high frequency of clinically insignificant positive SPT to maize in lettuce-allergic patients with sensitization to LTPs, indicating the presence of clinically irrelevant cross-reactive antibodies [25]. Likewise, Asero et al. [29] investigated a group of LTP-sensitized patients with multiple food allergies. While 21 of our 34 sera had IgE against carrot LTP, carrot was tolerated by all patients.

Most patients reported with grape allergy did not tolerate any grape species, wine or raisins [3–8]. Only 1 patient seems to have a grape allergy specific to a certain Americana blue grape but tolerance to other grapes [3]. Few patients had a history of atopic diseases and allergic symptoms to other food (nuts, apples, kiwis, peaches and eggs). In most cases, the diagnosis could demonstrate a positive prick-by-prick test and specific IgE to grape. Rodriguez et al. [6] found a 9.4-kDa protein that was recognized by IgE antibodies in their patients' sera. Interestingly, Pastorello et al. [9] recently identified endochitinase 4 (30 kDa), LTP Vit v 1 (9 kDa) and a thaumatin-like protein (24 kDa) as grape and wine allergens in 14 grape- and wine-allergic Italian patients.

Although sensitization to LTP in Germany was reported to be very rare [13], our data show that LTP sensitization exists in the northern parts of Europe, underlining the clinical relevance of LTP as a potentially dangerous food allergen.

Acknowledgment

We are grateful to Tanja Haase, Mechthild Retzek, Petra Pfeuffer and Petra Raith for excellent technical assistance.

References

- Ballmer-Weber BK: Lipid transfer protein as a potential panallergen? *Allergy* 2002;57:873–875.
- van Ree R: Clinical importance of non-specific lipid transfer proteins as food allergens. *Biochem Soc Trans* 2002;30:910–913.
- Bircher AJ, Bigliardi P, Yilmaz B: Anaphylaxis resulting from selective sensitization to Americana grapes. *J Allergy Clin Immunol* 1999;104:1111–1113.
- Giannoccaro F, Munno G, Riva G, Pugliese S, Paradiso MT, Ferrannini A: Oral allergy syndrome to grapes. *Allergy* 1998;53:451–452.
- Garcia-Robaina JC, Torre-Morin F, Sanchez-Machin I, Sanchez-Monge R, Barber D, Lombardero M: Anaphylaxis induced by exercise and wine. *Allergy* 2001;56:357–358.
- Rodriguez A, Trujillo MJ, Matheu V, Baeza ML, Zapatero L, Martinez M: Allergy to grape: A case report. *Pediatr Allergy Immunol* 2001;12:289–290.
- Vaswani SK, Chang BW, Carey RN, Hamilton RG: Adult onset grape hypersensitivity causing life threatening anaphylaxis. *Ann Allergy Asthma Immunol* 1999;83:25–26.
- Caiaffa MF, Tursi A, Macchia L: Grape anaphylaxis. *J Investig Allergol Clin Immunol* 2003;13:211–212.
- Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Fortunato D, Giuffrida MG, Perono GL, Calamari AM, Brenna O, Conti A: Identification of grape and wine allergens as an endochitinase 4, a lipid-transfer protein, and a thaumatin. *J Allergy Clin Immunol* 2003;111:350–359.
- Skin tests used in type I allergy testing. Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989;44(suppl 10):1–59.
- Vieths S, Schoning B, Petersen A: Characterization of the 18-kDa apple allergen by two-dimensional immunoblotting and microsequencing. *Int Arch Allergy Immunol* 1994;104:399–404.
- Sanz ML, Maselli JP, Gamboa PM, Oehling A, Dieguez I, de Weck AL: Flow cytometric basophil activation test: A review. *J Investig Allergol Clin Immunol* 2002;12:143–154.
- Scheurer S, Pastorello EA, Wangorsch A, Kastner M, Haustein D, Vieths S: Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. *J Allergy Clin Immunol* 2001;107:724–731.
- Schagger H, von Jagow G: Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;166:368–379.
- Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, Kraft D: Common epitopes of birch pollen and apples – Studies by Western and Northern blot. *J Allergy Clin Immunol* 1991;88:588–594.
- Scheurer S, Metzner K, Haustein D, Vieths S: Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. *Mol Immunol* 1997;34:619–629.
- Son DY, Scheurer S, Hoffmann A, Haustein 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;38:201–215.
- Pastorello EA, Farioli L, Pravettoni V, Giuffrida MG, Ortolani C, Fortunato D, Trambaioli C, Scibola E, Calamari AM, Robino AM, Conti A: Characterization of the major allergen of plum as a lipid transfer protein. *J Chromatogr B Biomed Sci Appl* 2001;756:95–103.
- Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, Baroglio C, Scibola E, Ansaloni R, Incorvaia C, Conti A: The major allergen of peach (*Prunus persica*) is a lipid transfer protein. *J Allergy Clin Immunol* 1999;103:520–526.
- Pastorello EA, D'Ambrosio FP, Pravettoni V, Farioli L, Giuffrida G, Monza M, Ansaloni R, Fortunato D, Scibola E, Rivolta F, Incorvaia C, Bengtsson A, Conti A, Ortolani C: Evidence for a lipid transfer protein as the major allergen of apricot. *J Allergy Clin Immunol* 2000;105:371–377.
- Diaz-Perales A, Collada C, Blanco C, Sanchez-Monge R, Carrillo T, Aragoncillo C, Salcedo G: Class I chitinases with hevein-like domain, but not class II enzymes, are relevant chestnut and avocado allergens. *J Allergy Clin Immunol* 1998;102:127–133.
- Schocker F, Luttkopf D, Scheurer S, Petersen A, Cistero-Bahima A, Enrique E, Miguel-Moncin M, Akkeraas J, van Ree R, Vieths S, Becker WM: Recombinant lipid transfer protein Cor a 8 from hazelnut: A new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004;113:141–147.
- Pastorello EA, Farioli L, Pravettoni V, Ispano M, Scibola E, Trambaioli C, Giuffrida MG, Ansaloni R, Godovac-Zimmermann J, Conti A, Fortunato D, Ortolani C: The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000;106:744–751.
- Diaz-Perales A, Tabar AI, Sanchez-Monge R, Garcia BE, Gomez B, Barber D, Salcedo G: Characterization of asparagus allergens: A relevant role of lipid transfer proteins. *J Allergy Clin Immunol* 2002;110:790–796.
- Miguel-Moncin M, Krail M, Scheurer S, Enrique E, Alonso R, Conti A, Cistero-Bahima A, Vieths S: Lettuce anaphylaxis: Identification of a lipid transfer protein as the major allergen. *Allergy* 2003;58:511–517.
- Guerbette F, Grosbois M, Jolliot-Croquin A, Kader JC, Zachowski A: Lipid-transfer proteins from plants: Structure and binding properties. *Mol Cell Biochem* 1999;192:157–161.
- Garcia-Ornelas F, Molina A, Segura A, Moreno M: The defensive role of nonspecific lipid-transfer proteins in plants. *Trends Microbiol* 1995;3:72–74.
- Pastorello EA, Vieths S, Pravettoni V, Farioli L, Trambaioli C, Fortunato D, Luttkopf D, Calamari M, Ansaloni R, Scibilia J, Ballmer-Weber BK, Poulsen LK, Wutrich B, Hansen KS, Robino AM, Ortolani C, Conti A: Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002;109:563–570.
- Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana CL, Verbeek E, Mohammadi T, Knul-Brettlova V, Akkeraas JH, Bulder I, Aalberse RC, van Ree R: 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.
- Pastorello EA, Ortolani C, Farioli L, Pravettoni V, Ispano M, Borga A, Bengtsson A, Incorvaia C, Berti C, Zanussi C: Allergenic 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.
- Coutos-Thevenot P, Jouenne T, Maes O, Guerbet F, Grosbois M, Le Caer JP, Boulay M, Deloire A, Kader JC, Guern J: Four 9-kDa proteins excreted by somatic embryos of grapevine are isoforms of lipid-transfer proteins. *Eur J Biochem* 1993;217:885–889.