# Component-resolved diagnosis with recombinant allergens in patients with cherry allergy

**Barbara K. Ballmer-Weber, MD,[a] Stephan Scheurer, PhD,[b] Philipp Fritsche, MD,[a] Ernesto Enrique, MD,[c] Anna Cistero-Bahima, MD,[c] Tanja Haase,[b] and Brunello Wüthrich, MD[a]** *Zurich, Switzerland, Langen, Germany, and Barcelona, Spain*

**Background: In pollen-related food allergy, extracts for skin prick tests (SPTs) are often not standardized, and the test reliability is affected by false-negative reactions.**
**Objective: We sought to evaluate a panel of recombinant allergens (RAs) derived from one allergenic food for use in component-resolved in vivo diagnosis, taking cherry as a model food.**
**Methods: Seventy-nine subjects were included in the study: 24 Swiss patients (group 1) with a positive double-blind placebo-controlled food challenge result to cherries, 23 patients with birch pollen allergy but without cherry allergy (group 2), 23 nonatopic subjects (group 3), and 9 Spanish patients with a history of a cherry allergy (group 4). SPTs were performed in duplicate by using recombinant cherry allergens (Bet v 1–related allergen: recombinant (r) Pru av 1; profilin: rPru av 4; and lipid transfer protein: rPru av 3) in concentrations of 10, 50, and 100 μg/mL. Furthermore, IgE reactivity to rPru av 1, rPru av 4, and rPru av 3 was assessed by means of immunoblot analysis.**
**Results: SPT responses with rPru av 1, rPru av 4, and rPru av 3 were positive in 92%, 17%, and 4% of the patients in group 1; in 74%, 30%, and 0% of the patients in group 2; in 0%, 22%, and 89% of the patients in group 4; and negative for all nonatopic subjects (group 3). Thus the sensitivity of a positive SPT response to at least one of the 3 RAs was 96%. The specificities, negative predictive values, and positive predictive values with the 3 RAs were 100%, 96%, and 100% if calculated in relation to the nonatopic control group but 17%, 79%, and 60% when calculated in relation to the control group with birch pollen allergy. The correlation between SPT and immunoblotting results was excellent. Sensitization to rPru av 3 was associated with more severe symptoms than sensitization to rPru av 1.**
**Conclusions: SPTs with RAs proved to be highly sensitive for diagnosis of cherry allergy. Component-resolved in vivo diagnosis with standardized amounts of stable RAs allows us to determine sensitization patterns directly, to correlate them with severity of clinical symptoms, and to analyze geographic differences. (J Allergy Clin Immunol 2002;110:167-73.)**

***Key words:*** *Food allergy, DBPCFC, cherry, recombinant allergens, diagnosis, skin prick testing, cross-reactivity*

Pollen-related food allergy is the most frequent form of food hypersensitivity in the adult population. However, diagnostic procedures, such as skin prick tests (SPTs) with food extracts and in vitro determination of specific IgE levels (RAST and CAP), currently used to assess the presence of a pollen related-food allergy are highly unsatisfactory.[1-7] SPTs with commercially available food extracts are often affected by false-negative reactions caused by a lack of standardization in regard to total protein content, content of single allergens, or biologic activity.[8,9] Moreover, even with well-prepared extracts, false-positive SPT responses do occur as a result of clinically insignificant cross-reaction.

Purified recombinant allergens (RAs) are currently available from different foods of plant origin, such as cherry,[10-12] celery,[13,14] apple,[15] and hazelnut.[16] They might be produced in suitable purity and batch consistency and hence might offer a perfectly standardized diagnostic material. These proteins are much more stable than antigens in food extracts because constituents of the plant matrix responsible for degradation (eg, polyphenoloxidase and proteases[4]) are absent. First results with rApi g 1 for skin testing were promising.[17]

Thus far, 4 cherry allergens have been identified. Pru av 1 and Pru av 4 share high amino acid sequence identity with the birch pollen allergens Bet v 1 and Bet v 2,[10,11] thus mediating the cross-reactivity observed between birch pollen and cherry. Furthermore, the cherry lipid transfer protein (LTP) Pru av 3[11] and a thaumatin-like protein, Pru av 2,[18] have been identified as allergenic proteins in cherries. Three of these 4 cherry allergens (rPru av 1, rPru av 3, and rPru av 4) have been cloned, sequenced, and characterized and applied in the present study to evaluate their skin test reactivity and, in parallel, their in vitro reactivity. Pru av 3 belongs to the family of nonspecific LTPs that have been identified as relevant food allergens in patients from Mediterranean countries.[19-21] Even though cross-reactivity to homologous structures has been described for mugwort[22] and *Parietaria* species pollen,[21,23] allergy to Rosaceae fruits was observed in the Mediterranean area independent of any pollen sensitization, suggesting that sensitization to LTPs might occur by the oral route.[19]

The aim of this study was (1) to validate diagnostic use of a panel of recombinant cherry allergens for skin prick testing in subjects with confirmed cherry allergy (ie, a

From [a]the Allergy Unit, Department of Dermatology, University Hospital, Zurich; [b]the Department of Allergology, Paul-Ehrlich-Institut, Langen; and [c]Institut Universitari Dexeus, Barcelona.

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Reprint requests: Barbara K. Ballmer-Weber, MD, Allergy Unit, Department of Dermatology, University Hospital Zürich, Gloriastr. 31, CH-8091 Zurich, Switzerland.



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*Abbreviations used*
DBPCFC: Double-blind placebo-controlled food challenge
LTP: Lipid transfer protein
NPV: Negative predictive value
OAS: Oral allergy syndrome
PPV: Positive predictive value
RA: Recombinant allergen
SPT: Skin prick test

positive double-blind placebo-controlled food challenge [DBPCFC] result), (2) to determine the specificity of skin testing with RAs by including patients with birch pollen allergy but no cherry allergy and nonatopic subjects as control populations, (3) to study the correlation of in vivo and in vitro diagnostic use of RAs, and (4) to assess geographic differences in sensitization pattern by including a group of patients with cherry allergy from Spain.

## METHODS

### Patients

Seventy-nine subjects were included in the study: 24 patients with a history of allergic reactions to cherry and a positive DBPCFC result (group 1), 23 patients with rhinoconjunctivitis pollinosa during birch pollen season but without cherry allergy (ie, with a negative open provocation result [group 2]), and 23 nonatopic control subjects (group 3) were recruited at the Allergy Unit of the University Hospital Zurich. Furthermore, 9 patients from the Institut Universitari Dexeus, Barcelona, with a clear-cut history of a cherry allergy (group 4) were included. Symptoms and time course of pollinosis were assessed in each patient.

### Ethical considerations

The study was reviewed and approved by the local ethical committee. All subjects provided written informed consent before enrollment in the study.

### Skin prick tests

SPTs were performed on the flexor aspect of the forearm with a standardized prick needle (Stallerpoint; Stallergènes, Antony, France), applying 25 μL of each test solution. Histamine dihydrochloride (10 mg/mL) was used as a positive control, and the glycerol-containing diluent of the prick solution (Soluprick; ALK, Hørsholm, Denmark) was used as a negative control. Patients were tested in duplicate with recombinant (r) Pru av 1, the cherry profilin rPru av 4, the cherry LTP rPru av 3 in dilution (100, 50, and 10 μg/mL), a self-produced cherry extract, and birch pollen extract (Soluprick, ALK). A subset of patients were tested with a commercially available cherry extract (Lofarma, Milano, Italy). Furthermore, SPTs were performed with pollen extracts from grass and mugwort (Soluprick, ALK) and in the Spanish patients (group 4) in addition with extracts from plane (Bial-Aristegui, Bilbao, Spain) and *Parietaria* pollen (Soluprick, ALK). Areas of wheal-and-flare reactions were recorded after 15 minutes, scanned, and calculated[24] with a software program (Archicad; Graphisoft R&D Rt, Budapest, Hungary). A wheal size of 7 $mm^2$ or greater was regarded as positive.[25]

### In vitro diagnosis

Specific IgE levels to birch pollen, rBet v 1, and rBet v 2 were measured with the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden).

### DBPCFCs with cherry

DBPCFCs were performed in patients with cherry allergy (group 1) by means of a 2-step spit (local mucosal challenge) and swallow procedure, as previously described for celery and carrot.[1,2] Two different drinks, identical in color, texture, and taste, were prepared according to the recipe suggested by the interest group of food allergy of the European Academy of Allergy and Clinical Immunology on their home page (www.ig-food.org), with minor modifications. The active drink contained 75 g of pitted cherries, 15 g of wheat flour, 10 g of cabbage, 6 teaspoons of mint syrup, 15 g of cocoa, a pinch of saffron, and 135 g of water mixed in a blender. The placebo drink contained the same ingredients but no cherries and, in addition, 13 g of sugar and 27 g of beetroot juice. Apart from cherries, all ingredients were known to be tolerated by each patient.

### Open provocation with cherries

In patients with birch pollen allergy but without cherry allergy and nonatopic control subjects, an open challenge was performed. These patients had to chew and swallow 6 fresh cherries.

### Protein extracts and RAs

Cherry extract was prepared from raw cherries (strain Schneiders), as described for apple.[26] Freeze-dried and redissolved extracts were kept at –20°C until used. Cloning and purification of the cherry allergens Pru av 1,[10] Pru av 3,[11] and Pru av 4[12] were performed as described recently. Purity of the allergens was confirmed by means of SDS-PAGE and Coomassie staining. The RAs were purified by using an endotoxin-removing column (Detoxi-Gel Endotoxin Removing Gel; Pierce, Rockford, Ill) to ensure the absence of endotoxins. The presence of pyrogenic substances was controlled by means of Limulus assay (Pyroquant; Pyroquant Diagnostik GmbH, Walldorf, Germany). Protein concentration was determined by using a commercial dye-binding assay (Roth, Karlsruhe, Germany) on the basis of the method described by Bradford.[27]

### Electrophoresis

Recombinant cherry allergens rPru av 1, rPru av 3, and rPru av 4 were separated by means of SDS-PAGE according to the method of Laemmli[28] by using a Bio-Rad (Munich, Germany) Mini Protean cell. The 15% separating gel was overlaid with a 5% stacking gel. Recombinant Pru av 4 was reduced with 1,4-dithiothreitol (Sigma-Aldrich, Deisenhofen, Germany), and rPru av 1 and rPru av 3 were applied under nonreducing conditions at a concentration of 0.5 μg of RA/cm.

### Immunoblotting

The proteins were transferred onto 0.2 μm of nitrocellulose membranes by means of tank blotting. The nitrocellulose membrane was blocked twice in 50 mmol/L Tris(hydroxymethyl)aminomethane/HCl buffer (pH 7.4) containing 0.15 mol/L sodium chloride and 0.3% Tween 20 (TBST).[29] Nitrocellulose strips were incubated overnight with 100 μL of patient sera and control sera of nonallergic subjects in 500 μL of TBST containing 0.1% BSA. IgE antibody detection was performed with alkaline phosphatase–conjugated mouse anti-human IgE (1:750 for 4 hours; PharMingen, San Diego, Calif). Antibody-bound proteins were visualized with the AP Conjugate Substrate Kit (Bio-Rad).

### Data analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated according to the method of Goldman,[30] according to previous publications.[1,3]

A $\phi$ coefficient was calculated with values between –1 and 1.[31] The Kolmogorov-Smirnov test was used to compare the sizes of the

wheals between group 1 (patients with cherry allergy) and group 2 (patients with birch pollen allergy but without cherry allergy).[31]

## RESULTS

### Patients

Forty-five female and 34 male subjects entered the study. The mean age of subjects included in group 1 was 29 ± 7 years (range, 21-47 years), that in group 2 was 30 ± 11 years (range, 17-60 years), that in group 3 was 31 ± 4 years (range, 24-39 years), and that in group 4 was 26 ± 8 years (range, 8-35 years). Case histories in respect to cherry allergy (group 1 and group 4) are summarized in Table I.

All patients in groups 1 and 2 reported pollinosis symptoms during the flowering season of birch, except for one patient of group 1 (no. 2) who had rhinitis during the flowering season of hazel. Seven of 9 Spanish patients, however, had pollinosis during the flowering season of plane (nos. 72, 74, and 76-79) and mugwort (no. 71).

### SPTs with RAs are highly sensitive for diagnosis of cherry allergy

In an initial screening a commercially purchased cherry extract produced a positive SPT response in just 20% of patients (n = 10) with a positive DBPCFC result to cherry, confirming the known poor quality of industrially manufactured fruit extracts.[32,33] This extract was replaced with a highly active low-temperature extract in which the presence of all known cherry allergens had been confirmed by means of IgE immunoblotting. This extract was used as a positive reference throughout the study.

Wheal areas of skin reactions to the recombinant cherry allergens rPru av 1, rPru av 3, and rPru av 4 and low-temperature cherry extract, respectively, are summarized in Table I for patients with cherry allergy (group 1 and 4) and in Table II for patients with birch pollen allergy but without cherry allergy (group 2).

*Group 1.* Twenty-two (92%) of 24 patients with positive DBPCFC results reacted to rPru av 1, 4 (17%) of 24 patients were sensitized to cherry profilin rPru av 4, and 1 (4%) patient was sensitized to cherry LTP (rPru av 3). SPT responses with the low-temperature extract were positive in 23 (96%) of 24 patients. All patients in group 1, except for patient 24, had positive SPT responses for birch pollen extract (96%); 18 (75%) of 24 patients had positive SPT responses to grass pollen (all except nos. 2, 3, 5, 16, 17, and 24); and 4 (17%) of 24 patients had positive SPT responses to mugwort pollen extract (nos. 11, 15, 18, and 24).

*Group 2.* Sixteen (70%) of 23 patients with birch pollen allergy but without cherry allergy had positive skin reactions to rPru av 1, and 7 (30%) of 23 had positive skin reactions to rPru av 4. All patients in group 2 had positive SPT responses to birch pollen (100%), 19 (83%) of 23 had positive SPT responses for grass pollen (all except nos. 25 and 32-34), and 7 (30%) of 23 had positive responses for mugwort pollen (nos. 26, 28, 36, 37, 40, 46, and 47).

*Group 3.* SPT responses with the recombinant cherry allergens, the cherry extract, and the pollen extracts were negative in all nonatopic control subjects.

*Group 4.* Eight (89%) of 9 of the Spanish patients had positive SPT responses for rPru av 3, and 2 (22%) patients were sensitized to rPru av 4. All patients of group 4 had negative SPT responses for rPru av 1 and birch pollen extract. Five (56%) of 9 patients reacted to plane pollen (nos. 71, 72, 76, 77, and 79), 2 (22%) of 9 reacted to grass pollen (nos. 72 and 74), and 3 (30%) of 9 reacted to mugwort pollen (nos. 71, 77, and 78), but no patients reacted to *Parietaria* pollen.

The Spanish patients were not challenged with cherries because they entered the study after the end of the cherry season. Therefore, we did not include them for the statistical analysis. The sensitivity of a positive SPT response to at least one of the 3 RAs was 96% in patients with true cherry allergy (ie, a positive DBPCFC result). The specificity, NPV, and PPV with the 3 RAs were 100%, 96%, and 100% when calculated in relation to the nonatopic control group but 17%, 79%, and 60% when calculated in relation to the control group with birch pollen allergy. We could not detect any statistically significant correlation between the skin reaction to any of the 3 recombinants (ie, size of the wheals) and the severity of clinical symptoms. However, when wheal sizes of patients with cherry allergy (group 1) were compared with those of patients with birch pollen allergy but without cherry allergy (group 2), statistically significant differences (Kolmogorov-Smirnov test) were observed for the SPTs with rPru av 1 at all concentrations used (100 μg/mL, $P < .05$; 50 μg/mL, $P < .005$; 10 μg/mL, $P < .05$) but not for SPT with rPru av 4 or rPru av 3.

### In vitro diagnosis

All patients in groups 1 and 2 showed increased specific IgE levels to birch pollen, and all patients were sensitized to rBet v 1, except patient 46 (group 2). Four patients in group 1 (nos. 4, 10, 18, and 22) and 8 patients in group 2 (nos. 31, 33, 36, 37, 43, 44, 46, and 47) had positive CAP results for rBet v 2. Two of 9 Spanish patients (group 4, nos. 72 and 74) were sensitized to birch pollen and rBet v 2 but not to rBet v 1.

### Food challenge with cherries

In the DBPCFCs 16 patients complained about symptoms strictly localized to the oral cavity (oral allergy syndrome [OAS]) at a mean provocation dose of 3.3 ± 2.4 g of cherries. OAS appeared during the local mucosa challenge (spit phase) in 10 patients and in 6 patients after swallowing 13 mL (n = 5) and 39 mL (n = 1) of the active drink, respectively.

In 7 patients, symptoms were not restricted to the oral cavity (Table I) and occurred at a mean provocation dose of 3.3 ± 3.53 g of cherries, in 5 patients during the local mucosa challenge, and in 2 patients after swallowing 39 mL of the active drink. One patient was not challenged (no. 24) because she had a severe allergic reaction with urticaria, angioedema, severe dyspnea, cough, and gastrointestinal symptoms accompanied by severe pain after cherry consumption.

Subjects in groups 2 and 3 did not experience any reaction during open provocation with fresh cherries.

**TABLE I.** Symptoms during DBPCFCs with cherries (group 1) and after cherry consumption according to patient history (groups 1 and 4), wheal area ($mm^2$) of skin reactions to recombinant cherry allergens and cherry extract, and immunoblot results with recombinant cherry allergens in patients with cherry allergy

| | Symptoms | | SPT cherry extract | SPT rPru av 1 | | | SPT rPru av 4 | | | SPT rPru av 3 | | | Immunoblot results | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient no. | History | DBPCFC | 1000 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | rPru av 1 | rPru av 4 | rPru av 3 |
| Group 1: positive DBPCFC results | | | | | | | | | | | | | | | |
| 1 | OAS | OAS | 9.8 | 25.6 | 10.5 | – | – | – | – | – | – | – | + | – | – |
| 2 | OAS | OAS | 11.8 | 8.1 | – | – | – | – | – | – | – | – | + | – | – |
| 3 | OAS | OAS, T | 27.8 | 21.5 | 22.7 | – | – | – | – | – | – | – | + | – | – |
| 4 | OAS, D | OAS | 12.3 | 31.4 | 42.3 | – | 52.15 | 47.5 | 29.7 | – | – | – | + | + | – |
| 5 | OAS, R | OAS | 26.85 | 26.7 | 37.9 | – | – | – | – | – | – | – | + | – | – |
| 6 | OAS, d | OAS, C | 69.55 | 50.3 | 53.1 | 13.6 | 31.65 | 28.5 | 12.25 | – | – | – | + | + | – |
| 7 | OAS | OAS | 9.25 | 37.0 | 27.4 | 13.2 | – | – | – | – | – | – | + | – | – |
| 8 | OAS | OAS | 14.1 | 40.6 | 30.1 | 20.75 | – | – | – | – | – | – | + | – | – |
| 9 | OAS | OAS | 33.9 | 54.6 | 24.1 | 27.6 | – | – | – | – | – | – | + | – | – |
| 10 | OAS | OAS | 26.1 | 56.3 | 71.4 | 27.65 | – | – | – | – | – | – | + | – | – |
| 11 | OAS, D | OAS, d | 47.2 | 65.1 | 53.5 | 18.85 | – | – | – | – | – | – | + | – | – |
| 12 | OAS | OAS | 54.5 | 71.9 | 102.4 | 33.45 | – | – | – | – | – | – | + | – | – |
| 13 | OAS | OAS | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 14 | OAS, D | OAS, T | 22.5 | 54.7 | 42.4 | 27.05 | – | – | – | – | – | – | + | – | – |
| 15 | OAS, g | OAS | 39.0 | 37.3 | 25.0 | 16.75 | – | – | – | – | – | – | + | – | – |
| 16 | OAS, D, g | OAS, g | 49.0 | 119.6 | 135.0 | 25.8 | – | – | – | – | – | – | + | – | – |
| 17 | OAS, D | OAS | 21.0 | 65.9 | 66.0 | 29.15 | – | – | – | – | – | – | + | – | – |
| 18 | OAS, D | OAS | 38.0 | 39.6 | 70.3 | 32 | 47.5 | 60.15 | 49.55 | – | – | – | + | + | – |
| 19 | OAS | OAS | 31.6 | 55.0 | 69.6 | 30.65 | – | – | – | – | – | – | + | – | – |
| 20 | OAS | OAS | 18.9 | 54.9 | 55.0 | 26 | – | – | – | – | – | – | + | – | – |
| 21 | OAS, g | OAS | 15.6 | 20.3 | 29.7 | 14.3 | – | – | – | – | – | – | + | – | – |
| 22 | OAS | OAS, T | 24.5 | 46.7 | 63.2 | 13.3 | 63.45 | 74.85 | 7.55 | – | – | – | + | + | – |
| 23 | OAS, D | OAS, D | 23.9 | 34.7 | 51.4 | 12.45 | – | – | – | – | – | – | + | – | – |
| 24 | U, A, D, C, g | ND | 39.3 | – | – | – | – | – | – | 76.25 | 49.75 | 10.4 | – | – | + |
| Mean | | | 27.8 | 42.4 | 45.1 | 15.9 | 8.1 | 8.8 | 4.1 | 3.2 | 2.1 | 0.4 | | | |
| SD | | | 16.4 | 25.8 | 32.2 | 12.2 | 19.1 | 21.3 | 11.7 | 15.6 | 10.2 | 2.12 | | | |
| Percentage* | | | 96 | 92 | 88 | 71 | 17 | 17 | 17 | 4 | 4 | 4 | | | |
| Group 4: positive history | | | | | | | | | | | | | | | |
| 71 | OAS | ND | 7.2 | – | – | – | – | – | – | 21.25 | 10.15 | – | – | – | + |
| 72 | OAS | ND | 28.0 | – | – | – | 53.15 | 32.6 | 29.2 | 82.75 | 30.6 | 12.95 | – | + | + |
| 73 | A | ND | 23.65 | – | – | – | – | – | – | 82.75 | 30.6 | 12.95 | – | – | + |
| 74 | OAS, A | ND | – | – | – | – | 12.8 | 8.1 | – | – | – | – | – | + | – |
| 75 | OAS, A, B | ND | 17.95 | – | – | – | – | – | – | 36.35 | 19.8 | 12.25 | – | – | + |
| 76 | A, R, D | ND | 25.3 | – | – | – | – | – | – | 45.85 | 28.45 | 10.7 | – | – | + |
| 77 | U, A, D | ND | 7.95 | – | – | – | – | – | – | 34.7 | 20.2 | – | – | – | + |
| 78 | OAS | ND | – | – | – | – | – | – | – | 22.95 | 19.1 | – | – | – | + |
| 79 | U, A, g | ND | 14.7 | – | – | – | – | – | – | 42.95 | 14.1 | – | – | – | + |
| Mean | | | 13.9 | – | – | – | 7.3 | 4.5 | 3.2 | 36.8 | 19.7 | 5.3 | | | |
| SD | | | 10.0 | – | – | – | 16.7 | 10.2 | 9.2 | 21.3 | 10.3 | 5.9 | | | |
| Percentage* | | | 78 | – | – | – | 44 | 44 | 33 | 89 | 89 | 44 | | | |

–, Negative result; *T,* thoracic oppression; *D,* dyspnea; *R,* rhinitis; *d,* dysphagia; *C,* cough; *g,* gastrointestinal symptoms; *U,* urticaria; *A,* angioedema; *ND,* not done; *B,* drop in blood pressure.
*Percentage of patients with a positive SPT response to the respective allergen and concentration.



## Immunoblot analysis and SPTs with RAs show excellent correlation

IgE immunoblot analysis of rPru av 1, rPru av 3, and rPru av 4 was performed with sera of all 79 subjects included in the study. Sera of all nonatopic control subjects (group 3) did not depict any IgE reactivity to the recombinant cherry allergens. Results of SPTs with recombinant cherry allergens and the immunoblot results with RAs showed an excellent correlation, with a $\phi$ coefficient of 0.95 for rPru av 1 and a $\phi$ coefficient of 1.0 for rPru av 3 and rPru av 4, respectively.

**TABLE II.** Wheal area ($mm^2$) of skin reactions to recombinant cherry allergens and cherry extract and immunoblot results with recombinant cherry allergens in patients with birch pollen allergy but without cherry allergy (group 2)

| | SPT cherry extract | SPT rPru av 1 | | | SPT rPru av 4 | | | SPT rPru av 3 | | | Immunoblot results | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient no. | 1000 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | 100 μg/mL | 50 μg/mL | 10 μg/mL | rPru av 1 | rPru av 4 | rPru av 3 |
| Group 2 | | | | | | | | | | | | | |
| 25 | 8.05 | – | – | – | – | – | – | – | – | – | – | – | – |
| 26 | 92.65 | 123.35 | 85.85 | 38.25 | 69.7 | 65.25 | 18.1 | – | – | – | + | + | – |
| 27 | 27.45 | 83.1 | 58.4 | 28.4 | – | – | – | – | – | – | + | – | – |
| 28 | 21.2 | 85.15 | 57.85 | 24.35 | – | – | – | – | – | – | + | – | – |
| 29 | 31.05 | 52.8 | 23.15 | 9.85 | – | – | – | – | – | – | + | – | – |
| 30 | 8.02 | 11.75 | 9.75 | – | – | – | – | – | – | – | + | – | – |
| 31 | – | 20.7 | 24.45 | 10.4 | – | – | – | – | – | – | + | – | – |
| 32 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 33 | 17.06 | 64.0 | 14.1 | 20.95 | 63.1 | 43.75 | 26.75 | – | – | – | + | + | – |
| 34 | – | 7.25 | 7.1 | – | – | – | – | – | – | – | + | – | – |
| 35 | – | 13.05 | 10.85 | – | – | – | – | – | – | – | + | – | – |
| 36 | 18.01 | – | – | – | 69.55 | 35.95 | 24 | – | – | – | – | + | – |
| 37 | – | 17.55 | 8.05 | – | 41.5 | 57.4 | – | – | – | – | + | + | – |
| 38 | 9.95 | 13.4 | 12.15 | – | – | – | – | – | – | – | + | – | – |
| 39 | 15.95 | 50.95 | 42.65 | 16.8 | – | – | – | – | – | – | + | – | – |
| 40 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 41 | 8.05 | 9.95 | – | – | – | – | – | – | – | – | + | – | – |
| 42 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 43 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 44 | 10.04 | 22.9 | 16.75 | 9.45 | 20.35 | 18.8 | 8.5 | – | – | – | + | + | – |
| 45 | – | 47.5 | 16.8 | – | 58.0 | 90.2 | 41.45 | – | – | – | + | + | – |
| 46 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 47 | 53.55 | 64.05 | 38.05 | 24.75 | 109.4 | 84.7 | 21.25 | – | – | – | + | + | – |
| Mean | 14.0 | 29.9 | 18.5 | 8.0 | 18.8 | 17.2 | 6.1 | – | – | – | | | |
| SD | 21.7 | 34.8 | 23.2 | 11.7 | 32.4 | 29.9 | 11.7 | – | – | – | | | |
| Percentage* | 57 | 70 | 65 | 39 | 30 | 30 | 26 | – | – | – | | | |

–, negative result.
*Percentage of patients with a positive SPT response to the respective allergen and concentration.

## DISCUSSION

Over the last 10 years, approximately 30 studies have been performed with RAs for skin testing.[34,35] Most of these studies dealt with perennial allergens, such as mites,[36] with fungi[37] or with pollen, especially birch pollen.[38-40] In pollen-related food allergy, however, up to now there has been very limited experience regarding the use of RAs for diagnostic purposes.[17,41] For the present study, we have chosen cherry as a model of an allergenic food derived from the Rosaceae family. For the first time, in vivo and in vitro testing was performed with a panel of 2 pollen-related recombinant food allergens and one non–pollen-related food allergen with DBPCFCs as a reference method for positivity. Because clinically insignificant cross-reactivity is a main problem in pollen-related food allergy, a group of birch pollen-sensitized patients with negative open challenge results to cherry was also included.

Both the DBPCFC and SPT procedures proved to be safe for the patients, and the SPTs with the panel of RAs perfectly matched with SPT results obtained with a highly active low-temperature extract prepared in our laboratory. The sensitivity of a positive SPT response to at least one of the 3 RAs was 96% equivalent to the sensitivity of SPTs with our self-prepared cherry extract. By contrast, a commercially purchased cherry extract was found to be unsuitable to detect sensitization in 8 of 10 patients with cherry allergy. The latter result is most likely the result of degradative processes caused by endogenous enzyme activities. We have observed similar discrepancies between the sensitivity of commercially available and self-prepared extracts in former studies on allergies to other plant foods.[1-3] Prick-to-prick test results with native cherries were positive in all but one patient with true cherry allergy (sensitivity of 96%, results not shown). Even though this diagnostic approach was as sensitive as the application of our 3 RAs or the self-prepared cherry extract, one has to consider that the use of fresh food for SPTs is not standardized at all.

Ninety-two percent of the 24 patients with cherry allergy in group 1 had a positive SPT response to rPru av 1, 17% had a positive response to rPru av 4, and 1 (4%) subject with an anaphylactic reaction after cherry consumption to rPru av 3. A similar IgE prevalence for the recombinant cherry allergens has been reported in an in

vitro study performed by Scheurer et al[11] in German patients with cherry allergy selected on the basis of a positive case history.

Cherry extract immunoblots were performed with sera of all patients with positive DBPCFC results to investigate the potential role of cherry allergens not available as recombinant proteins (not shown). Ninety-two percent of the sera showed IgE binding on immunoblots. Extract immunoblotting revealed that all patients' sera positive to one of the recombinant allergens reacted in the molecular weight range between 9 kd and approximately 18 kd, corresponding to Pru av 1, Pru av 3, and Pru av 4. In addition, weak IgE reactivities were observed in the upper molecular weight range (>50 kd), most likely representing cross-reactive carbohydrate determinants. No IgE reactivity was found against a putative 23-kd allergen, which has been described by Inschlag et al[18] as a thaumatin-like protein. Thus, at least in Swiss patients with cherry allergy, thaumatin Pru av 2 does not fulfill the criterion of a major allergen.

We could not detect any statistically significant correlation between the skin reaction to any of the 3 recombinants (ie, size of the wheals) and the severity of clinical symptoms. However, by including 9 patients with cherry allergy from Barcelona, Spain (group 4), we observed an impressive geographic difference of the sensitization pattern to the 3 RAs. Even though we have included the Swiss patients on the basis of a positive case history concerning cherry and not birch pollen allergy, all were sensitized to birch pollen, as determined by means of CAP assay. In the Spanish study population, however, only 2 patients sensitized to grass pollen showed specific IgE to birch pollen profilin Bet v 2, most likely as a result of cross-reactivity with grass profilin. Accordingly, 92% of the Swiss patients, but none of the Spanish patients, were sensitized to the Bet v 1–related cherry allergen rPru av 1. In the Swiss study population with cherry allergy, however, only one patient (no. 24) was sensitized to the cherry LTP rPru av 3, but 8 of 9 Spanish patients were thus sensitized. Two Spanish patients (nos. 73 and 75) who did not have pollinosis were monosensitized to rPru av 3. This constellation suggests a primary sensitization to LTP in these 2 patients.

More importantly, our data strengthen the view of Bet v 1–related proteins being mild and LTP representing potentially severe fruit allergens: 55% (10/18) of patients monosensitized to rPru av 1 had symptoms strictly localized to the oral cavity (OAS) compared with 25% (2/8) of patients monosensitized to rPru av 3. Sixty-six percent (6/8) of patients exclusively sensitized to rPru av 3, however, reacted with urticaria, angioedema, or both, symptoms not observed in any of the patients monosensitized to rPru av 1. In general, the clinical manifestation of patients sensitized to cherry LTPs was more severe than that in patients sensitized to rPru av 1. This is in accordance with previous reports suspecting that a sensitization to LTPs might be accompanied by a higher prevalence of systemic symptoms.[20,42]

Because the immunologic basis of pollen-related food allergy is IgE cross-reactivity between pollen and food allergens of plant origin, patients with pollinosis but no food allergy might nevertheless have a positive SPT response or CAP result to fruits and vegetables. To investigate the effect of clinically insignificant cross-reactions on in vitro and in vivo diagnosis of fruit allergy, we have included 3 different control populations for assessing the specificity, PPV, and NPV of SPTs with RAs: Control group 1 consisted of patients with birch pollen allergy but without cherry allergy, as determined by a negative open provocation result, and control group 2 consisted of nonatopic subjects. The specificity, NPV, and PPV with the 3 RAs were excellent (100%, 96%, and 100%) when calculated in relation to the nonatopic control group but relatively poor (17%, 79%, and 60%) when calculated in relation to the control group with birch pollen allergy. From these results, we conclude that recombinant cherry allergens do not elicit unspecific reactions, such as irritative skin reactions, because none of the nonatopic control subjects showed any reaction to the 3 cherry allergens tested. Second, our data in patients of group 2 clearly demonstrate that neither SPT nor in vitro results might indicate whether sensitization to cherry in patients with birch pollen allergy might represent clinically manifest allergy or clinically irrelevant sensitization because IgE from patients with birch pollen allergy cross-react with Bet v 1– and Bet v 2–related epitopes on rPru av 1 and rPru av 4. Although the mean wheal areas to Pru av 1 and cherry extract were lower in group 2 compared with those in group 1 ($P < .05$), there was no threshold wheal area predicting food allergy in individual subjects. The factors determining clinical significance of cross-reactive IgE responses remain unclear.

We conclude that SPTs with 3 RAs proved to be safe and highly sensitive for the diagnosis of cherry allergy. The sensitivity of SPTs with this highly standardized diagnostic material was equivalent to the sensitivity of SPTs with a cherry extract prepared by means of a complex low-temperature method[26] and clearly superior to that with a commercial cherry extract. Component-resolved in vivo diagnosis with standardized amounts of stable RAs allows on-site determination of sensitization patterns to defined allergens in clinical practice. These patterns can be correlated to severity of clinical symptoms and used to analyze geographic differences.

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