

Severe Allergy to Sharon Fruit Caused by Birch Pollen

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Key Words

Allergy · Bet v 1 · Food · Profilin · Sharon fruit

Abstract

Background: Allergy to sharon fruit (persimmon) has been only rarely reported. Cross-reactivity with pollen (profilin and Bet v 6) appeared to be involved, but Bet v 1 has not been implicated previously. **Objective:** It is our aim to identify whether Bet v 1 sensitization is linked to sharon fruit allergy. **Methods:** Two patients with a reaction upon first exposure to sharon fruit were included in the study, as well as 7 patients with birch-pollen-related apple allergy. Sensitivity was assessed by skin prick testing (SPT), a radio-allergosorbent test (RAST) and immunoblotting. RAST analysis was performed for Bet v 1, Bet v 2 and Bet v 6. Cross-reactivity was evaluated by RAST and immunoblot inhibitions. Biological activity of IgE was measured by basophil histamine release. Sharon fruit allergy was evaluated by double-blind placebo-controlled food challenge (DBPCFC) or open challenge (OC). **Results:** Both sharon-fruit-allergic patients demonstrated positive reactions in the RAST (8.6 and 6.2 IU/ml, respectively) and SPT (wheal area 37 and 36 mm²). Sharon fruit allergy was confirmed by DBPCFC in 1 patient.

The second patient refused a challenge because of the severe initial reaction. Sera from both patients were reactive to Bet v 1 and Bet v 6, which was cross-reactive with sharon fruit by inhibition assays. The patient with the severest reactions was reactive to profilin on immunoblotting. However, profilin did not induce significant histamine release, nor did Bet v 6. Bet v 1 induce approximately 60% histamine release. An OC with sharon fruit in 7 patients allergic to birch pollen and apple, who had not eaten sharon fruit previously, was positive in 6/7 cases. **Conclusions:** Birch-pollen-related allergy to sharon fruit is mediated by the known cross-reactive pollen allergens including Bet v 1 and may become more of a problem should sharon fruit consumption increase.

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Introduction

IgE-mediated food allergy is caused by primary sensitization to pollen [1] or latex [2], resulting in cross-reactive IgE antibodies to a variety of foods, or by primary sensitization to food.

In this study, we report 2 cases of allergy to sharon fruit. Sharon fruit is the edible fruit of the persimmon tree

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1018–2438/05/1361–0045\$22.00/0

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that belongs to the Ebenaceae family. Once native to China and Japan, persimmon trees were introduced to Europe and California in the mid-19th century; the fruit is currently produced in California where it is known as persimmon, and in France, Italy and Spain where it is known as kaki [3]. A study by Anliker et al. [4] on sharon fruit allergy suggested pollen profilin and cross-reactive carbohydrate determinants (CCD) as sources of cross-reactive allergens. In addition, Karamloo et al. [5] demonstrated the involvement of a Bet v 6-related food allergen (phenylcoumaran benzylic ether reductase) as cross-reactive allergen in sharon fruit. So far, the major birch pollen allergen Bet v 1 was not identified as a possible cause of cross-reactive sharon fruit allergy.

Bet v 1 accounts for most of the *in vitro* (serologic) and *in vivo* (clinical) cross-reactivity with Rosaceae fruits (e.g. apple, peach, pear) and to a lesser extent with Umbelliferae vegetables (e.g. celery, carrot, fennel) [6, 7]. Food allergy in these patients is generally mild and mostly limited to the oral cavity – the so-called oral allergy syndrome (OAS) [8] – and sometimes associated with mild to moderate angioedema. Some patients also develop rhinoconjunctivitis and mild asthmatic symptoms due to the fact that food homologs of Bet v 1 do not survive the harsh acidic and proteolytic environment of the gastrointestinal tract. More recently, however, Bet v 1-related allergens in carrot and celery and also in soy have been implicated in severe allergic symptoms as well [9, 10]. Although serological observations support a role for some Bet v 1 homologs in severe symptoms, increased stability has not been proven.

Profilin is a 12- to 15-kDa monomeric actin-binding protein present in all eukaryotic cells. It was first reported as a minor allergen in birch pollen, but is a ubiquitous plant panallergen and one of the main causes of cross-sensitization between pollen and plant-derived foods. Sera from patients with pollen allergy sensitized to profilin show IgE cross-reactivity with fruits and vegetables. Profilin has been implicated in the birch-Rosaceae fruit and the birch-mugwort-celery-spice syndrome [11, 12], and several studies concluded that this protein can also play a role in patients allergic to a variety of other foods [6, 11, 13–21]. Results of recent studies suggest that IgE antibodies to profilin in foods have little or no clinical relevance [22–24]. Other publications have clearly demonstrated a close correlation between food allergy and IgE antibodies to profilin. For example, Rodriguez-Perez et al. [25] reported a dominant role for profilin in Spanish grass-pollen-allergic patients with clinical peach allergy. Most likely, this discrepancy is caused by selection bias.

In the former studies, pollen allergy with cross-reactive IgE antibodies was used as selection criterion, whereas clinical fruit allergy was used in the latter studies.

More recently, Bet v 6 (phenylcoumaran benzylic ether reductase) was identified as a cross-reactive allergen between birch pollen and plant foods [5].

The aim of our study was to confirm sharon fruit allergy by double-blind placebo-controlled food challenge (DBPCFC) and to investigate whether birch pollen allergens, in particular Bet v 1, were involved in sharon fruit allergy, in addition to Bet v 2 and Bet v 6.

Material and Methods

Patients

Two adult patients visited the outpatient clinic of the University Medical Center Utrecht, reporting a reaction to sharon fruit upon first ingestion. Seven typical birch-pollen- and apple-allergic patients of the University Medical Center Utrecht were recruited for open food challenges (OCs) with sharon fruit. None of them had ever eaten sharon fruit prior to the OC. All patients were apple allergic and had seasonal rhinitis during the birch pollen season in the Netherlands (March to May) and a positive skin test (>7 mm²) and radio-allergo-sorbent test (RAST; >0.7 kU/ml) for birch pollen and apple.

The study was reviewed and approved by the local ethical committee. Informed consent was obtained before enrolment in the study.

Skin Prick Tests

Skin prick tests (SPTs) were performed on the flexor aspect of the forearm with a standardized prick needle (ALK Lancet) and documented according to Dreborg and Frew [26]. Histamine dihydrochloride (10 mg/ml) was used as a positive control, and the glycerol diluent of the SPT extracts was used as a negative control (ALK-Abelló, Nieuwegein, The Netherlands). Patients underwent SPTs with commercial extracts for birch, grass and olive pollen, celery, hazelnut, mugwort, Parietaria, peanut, ragweed and walnut (ALK-Abelló) and with fresh apple, peach and sharon fruit (prick-to-prick method). Reactions were measured after 15 min by copying the wheal reaction with transparent adhesive tape onto a record sheet. The skin wheal areas were determined by computer scanning [27]. SPTs were regarded positive when the area was at least 7 mm².

Double-Blind Placebo-Controlled Food Challenges/Open Challenges

Clinical reactivity to sharon fruit was investigated by DBPCFC in the sharon-fruit-allergic patients who had reported a mild adverse reaction to this food. DBPCFC was carried out using a procedure which has been described elsewhere [28]. Due to the known lability of Bet v 1-related food allergens, the challenge meals containing 5, 10 and 25 g of fresh shredded sharon fruit were prepared within 5 min before administration. OCs were performed with 2 samples (5 and 10 g) of fresh sharon fruit. The same method of scoring was used and the same precautions were taken as in the case of DBPCFC.

Table 1. Clinical history, SPT and DBPCFC results of case reports 1 and 2

Patient No.	Age, year	Sex	Rhino-conjunctivitis	Asthma	Food allergy	Positive SPT results (wheal size in mm ²)	Symptoms upon ingestion	DBPCFC	Treatment after DBPCFC or after ingestion
1	35	F	birch and grass	no	ap, pe, ha, ba, sf	histamine (32), bi (38), sf (36), ap (57), pe (43), pea (21), ha (42), ba (25)	OAS (itching and swelling of the lips, mouth, throat), dyspnea, tachycardia, nausea, cramps	not willing	antihistamine and prednisone injections
2	31	M	birch and grass	no	ap, pea, ha, sf	histamine (37), bi (43), gr (47), sf (37), ap (69), pe (76), pea (113), ha (38), ce (48), phl (12), ol (20)	OAS	OAS (60 VAS)	antihistamine

Only positive SPT results > 7 mm² are shown. ap = Apple; ba = banana; bi = birch pollen; sf = sharon fruit; ha = hazelnut; ol = olive pollen; par = Parietaria; pe = peach; pea = peanut; phl = *Phleum pratense*; VAS = visual analogue scale.

Extracts and Allergens

Sharon fruit was obtained from a local food store. Extracts were prepared essentially according to Björkstén et al. [29]. In brief, the fruit (without core) was homogenized at 10% w/v in 0.1 mol/l phosphate buffer pH 7.0, containing 2% polyvinylpyrrolidone, 7 mmol/l diethyldithiocarbamate, 2 mmol/l EDTA and 2.6 mmol/l NaN₃. After stirring for 1 h, particulate matter was removed by centrifugation at 18,000 g for 30 min. The supernatant was dialyzed against distilled water and frozen in aliquots. Other food extracts were prepared as described elsewhere [29]. Birch pollen extract (BPE) was made as described before [30]. Recombinant (r)Bet v 2 was purchased from Biomay (Vienna, Austria), natural (n)Bet v 1 and nMal d 1 were purified by affinity purification using monoclonal antibody 5H8 (directed to nBet v 1 and homologs) [31]. rBet v 6 was produced as described elsewhere [5]. For application in RAST, 4 mg extract or 100 µg purified allergen was coupled to 100 mg of CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech, Uppsala, Sweden).

RAST and RAST Inhibition

RAST was performed as described previously [32, 33]. Results were expressed as international units IgE per ml (IU/ml). Calculation was performed by means of a standard curve that was obtained by RAST with a dilution series of a chimeric monoclonal IgE antibody against the major house dust mite allergen Der p 2 and Sepharose-coupled recombinant Der p 2 [34]. A RAST value >0.3 IU/ml was regarded as positive. For RAST inhibition, serum was preincubated with (serial) dilutions in 50 µl phosphate-buffered saline albumin, 0.1% Tween-20 (PBS-AT) of the inhibitor – nMal d 1, rBet v 2, nBet v 1, rBet v 2, rBet v 6, BPE or sharon fruit extract (SFE) – prior to the addition of Sepharose-coupled SFE; this was incubated between 30 min and 2 h. For the uninhibited value, serum was preincubated with 50 µl PBS-AT. Subsequent steps were identical to those described for the RAST. Results were expressed as percentage inhibition.

SDS-PAGE/Immunoblotting

Proteins were separated by SDS-PAGE (NuPAGE®, Invitrogen, Carlsbad, Calif., USA; 4–12% Bis-Tris gel, protein 10 µg/cm) according to the protocol of the manufacturer and silver stained using the

protein silver staining kit (Bexel Biotechnology, Union City, Calif., USA).

Western blotting was performed by transferring the proteins after electrophoresis separation (semidry) to nitrocellulose on a Novablot electrophoretic transfer apparatus, according to the protocol of the manufacturer (Invitrogen). After blocking with PBS-A (PBS, 10 mM EDTA, 0.3% BSA) for a minimum of 10 min, the blots were cut into strips prior to immunoprobings overnight with 150 µl human serum in 3 ml of PBS-AT. After washing away unbound serum with PBS-T (PBS, 0.1% Tween-20), radiolabeled sheep antibodies against human IgE (Sanquin, Amsterdam, The Netherlands) were used for detection of bound IgE. Blots were exposed to X-ray film (Eastman Kodak Company, Rochester, N.Y., USA). For blot inhibition studies, 150 µl of the inhibitor was preincubated for 30 min together with the patient serum. Incubation of the blot strips and detection were performed as above.

Basophil Histamine Release Assay

White blood cells were isolated from the blood of a nonallergic donor by Percoll (Amersham) centrifugation and stripped of IgE by lactic acid treatment as described elsewhere [35, 36]. Subsequently, cells were resensitized with the patient's serum. Histamine release was performed with BPE and SFE (1 ng/ml–100 µg/ml) and with purified nBet v 1, rBet v 6 or rBet v 2 (100 pg/ml–10 µg/ml). Liberated histamine was measured by the fluorometric method as described by Siraganian [37]. Stripped cells were used as a negative control and induced a histamine release lower than 3% (data not shown).

The protocol was approved by the medical ethical committee of the Amsterdam Medical Center under project No. MEC97/030.

Results

Case Report 1

A 36-year-old female had rhinoconjunctivitis in the birch pollen season since early youth and increasingly experienced OAS (table 1). After eating sharon fruit for the first time, she noted OAS within 5 min. Twenty min-

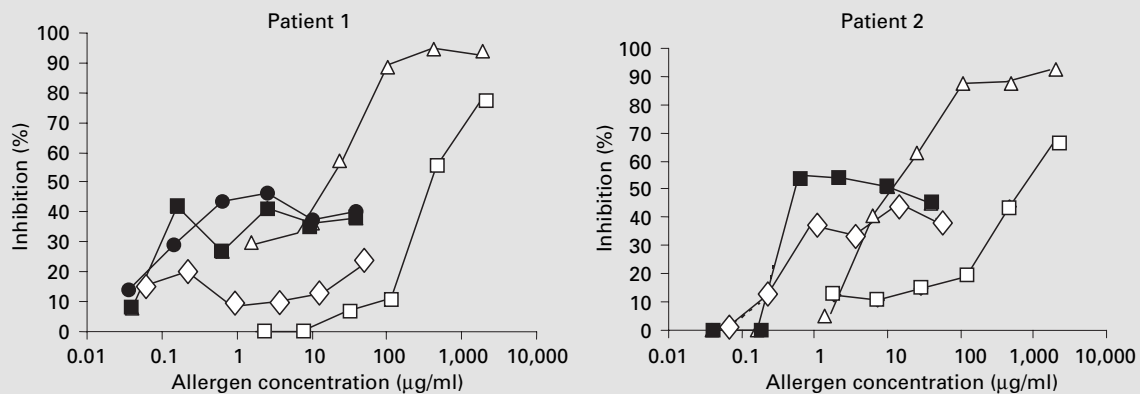


Fig. 1. Sharon fruit RAST inhibition of serum IgE of patients 1 and 2. Inhibitors: sharon fruit (□), BPE (△), nBet v 1 (■), rBet v 6 (◇) or rBet v 2 (●).

Table 2. Results of specific IgE determinations

	Patient 1	Patient 2
Birch pollen	20.1	35.9
Apple	12.8	21.4
Sharon fruit	8.6	6.16
Peach	9.3	5.6
Hazelnut	11.4	8.8
Peanut	8.7	9.7
nBet v 1	12.5	95.1
rBet v 2	2.7	ND
rBet v 6	1.8	6.1
CCD	0.2	0.8

Results are given in IU/ml. ND = Not detected.

utes later, she experienced cramps, nausea, itching and erythema of the neck, palms of the hands and feet. Moreover, dyspnea and swelling of the throat occurred. She was hospitalized and treated intravenously with antihistamines and prednisolone.

Case Report 2

A 31-year-old man with a history of rhinoconjunctivitis in the birch pollen season increasingly reported episodes of OAS (table 1). He developed OAS within 5 min

after eating a very small piece of fresh sharon fruit. He had never eaten this fruit before.

Confirmation of Sharon Fruit Sensitization and Allergy by SPT, RAST and DBPCFC

Both patients demonstrated a positive SPT for sharon fruit (36 and 37 mm², respectively) and the other fruits and nuts to which they reported OAS (table 1). RAST analysis confirmed sensitization to sharon fruit (8.6 and 6.2 IU/ml, respectively) and the other foods (table 2). Due to the severity of the initial reaction, patient 1 refused to be challenged. For patient 2, allergy to sharon fruit was confirmed by DBPCFC (table 1). He reacted with itching in his mouth and throat after a relatively small dose (5 g) of sharon fruit. No reactions occurred to placebo.

Sharon Fruit Allergy Is Caused by IgE against Birch Pollen Allergens

To investigate whether the sharon fruit allergy was caused by allergens homologous to birch pollen allergens, sera of both patients were tested in a RAST on nBet v 1, rBet v 2 and rBet v 6 (table 2). Both patients recognized nBet v 1 and rBet v 6 (RAST). Only patient 1 had specific IgE against profilin as well. RAST inhibition (fig. 1) revealed strong cross-reactivity between birch pollen and sharon fruit. The sharon fruit RAST was inhibited by BPE (by >90%) at lower concentrations than by SFE, pointing towards birch pollen as the primary sensitizer. Partial inhibition was observed with nBet v 1, rBet v 2

Fig. 2. Immunoblot inhibition studies of serum IgE of patients 1 and 2. Lane 1: uninhibited serum; lane 2: 10 µg/ml rBet v 6; lane 3: 10 µg/ml nBet v 1; lane 3*: 10 µg/ml nBet v 2; lane 4: 4.4 mg/ml BPE; lane 5: 2.5 mg/ml SFE, and LC: label control.

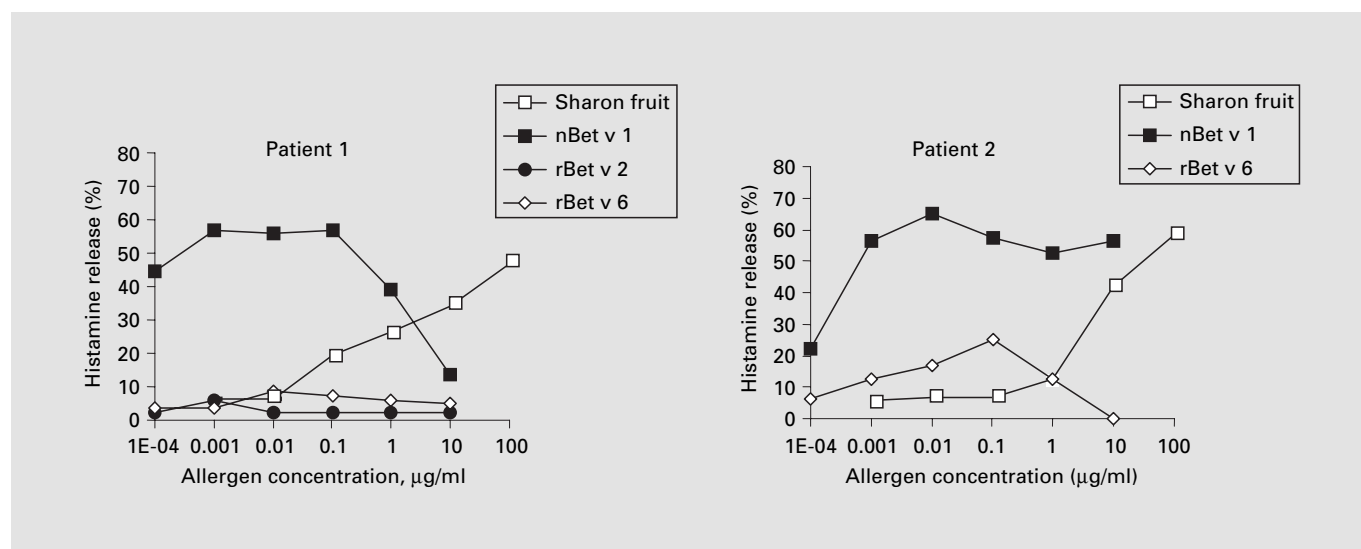
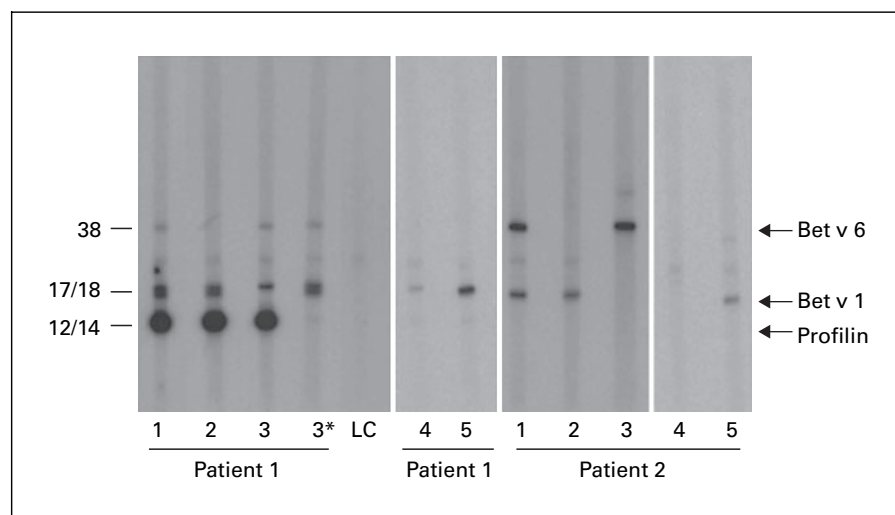


Fig. 3. Basophil histamine release assay with serum of patient 1 or 2. nBet v 1 (■), SFE (□), rBet v 6 (◇) and rBet v 2 (●, patient 1).

and rBet v 6 for patient 1 and with nBet v 1 and rBet v 6 for patient 2. By adding up percentages of inhibition achieved with individual allergens, complete inhibition was approached. Immunoblot (inhibition) analysis confirmed the presence of the 3 birch pollen homologs in sharon fruit (fig. 2). Both patients recognized bands around 17 and 38 kDa. The 17-kDa band was inhibited by the addition of nBet v 1, the 38-kDa band by rBet v 6. Both bands were inhibited by BPE and SFE. In addition, patient 1 recognized a band around 12–14 kDa, which

was inhibited by rBet v 2 and BPE or SFE. Furthermore, this patient recognized an additional band at 18 kDa which was not inhibited by the addition of nBet v 1, but was partially inhibited by BPE.

Biological Activity of Cross-Reactive IgE

To assess the biological activity of anti-sharon fruit and anti-Bet v 1, 2 and 6 IgE, histamine release tests were performed using sera from patients 1 and 2 (fig. 3). SFE induced up to 45 and 55% release, respectively. Bet v 1

Table 3. Specific IgE determination, SPT and OC results of patients 3–9

Patient No.	Birch IU/ml	Apple IU/ml	Sharon fruit IU/ml	Phleum IU/ml	nBet v 1 IU/ml	nBet v 2 IU/ml	rBet v 6 IU/ml	SPT mm ²	OC
3	8.9	3.1	0.5	1.7	16.1	0.1	0.4	10	pos.
4	4.9	1.1	0.7	11.6	5.4	0.2	0.2	36	pos.
5	29.8	9.6	1.9	0.5	61.0	0.3	1.6	39	pos.
6	3.8	1.6	0.4	0.3	4.9	0.3	0.3	24	pos.
7	9.6	1.3	1.2	3.4	12.7	0.3	1.1	29	pos.
8	3.3	1.6	1.8	3.0	4.0	0.1	0.4	19	pos.
9	4.8	0.5	0.6	19.8	2.1	0.6	0.5	9	neg.

Pos. = positive; neg. = negative.

resulted in >50% release at a concentration of 1 µg/ml. Bet v 2 and Bet v 6 did not have significant biological activity (<10%) in patient 1. Specific IgE from patient 2 against Bet v 6 had poor but statistically significant biological activity, i.e. <25% release at 100 µg/ml.

Sharon Fruit Allergy in Patients with Allergy Related to Birch Pollen and Apple

Seven adult patients (patients No. 3–9, 2 females and 5 males, mean age 35.2 years) with a convincing history of allergy to birch pollen and apple and a positive skin test (>7 mm²) for both allergens had a positive SPT to sharon fruit (table 3). All had significant but relatively low specific IgE titers to sharon fruit with a mean of 0.9 IU/ml and a range of 0.4–1.9 IU/ml. None of them had eaten sharon fruit previously. They were subjected to an OC with sharon fruit. Six of 7 patients reported OAS (itching in mouth and throat) already after ingestion of 5 g sharon fruit.

Discussion

Allergy to sharon fruit was first described by Prandini and Marchesi [38] and Martinez et al. [3]. The study of Anliker et al. [4] suggested a role for primary sensitization by grass pollen. They implicated profilin and CCD as the main allergens in sharon fruit. None of these studies linked sharon fruit allergy explicitly to birch pollen allergens.

In this study, we describe 2 patients with a birch-pollen-related food allergy with an adverse reaction to sharon fruit upon first contact with this fruit, which was severe in 1 case. This observation strongly suggested a role for birch pollen as primary sensitizer. Indeed, by RAST and immu-

noblot inhibition, cross-reactivity between sharon fruit and birch pollen was confirmed. While studying this cross-reactivity in more detail, a major role for Bet v 1 was demonstrated. In agreement with the findings of Anliker et al. [4], profilin appeared to be involved in 1 of the 2 patients as well. Furthermore, Bet v 6 was shown to be a cross-reactive allergen for both patients, as was earlier reported by Karamloo et al. [5].

Compared with Bet v 1, IgE titers against profilin and Bet v 6 were much lower. Furthermore, the biological activity of IgE antibodies against both profilin and Bet v 6, as assessed by the histamine release assay, was low or negligible, suggesting that these cross-reactive allergens are of limited clinical relevance to these patients. Poor biological activity has been reported for several cross-reactive structures including profilin and CCD [22, 39]. The only allergen that demonstrated clear biological activity in our study was Bet v 1.

Although we have convincingly shown that Bet v 1 plays an important role in birch pollen-sharon fruit cross-reactivity, its clinical relevance is still not completely clear. In general, Bet v 1 homologs in fruit are thought to be extremely sensitive to gastrointestinal proteolysis and, therefore, to induce only mild oral symptoms. However, the serology and biological activity tests performed for patient 1 suggest that the sharon fruit homolog of Bet v 1 is instrumental in the patient's severe sharon fruit allergy. Severe food allergy has been reported for some Bet v 1 homologs, like carrot (Dau c 1) [40], celery (Api g 1) [9] and soy (Gly m 4) [10].

Sharon fruit has only been recently introduced on the European market. This study has shown that it can be expected that the number of adverse reactions in birch-pollen-allergic patients will increase if consumption figures grow. Six out of 7 birch-pollen-allergic patients that

had not previously eaten this fruit had a positive OC. This is in line with a recent study in Sweden [41] where 397 birch-pollen-allergic patients were asked to fill in a questionnaire about possible reactions to a number of exotic fruits that they had only recently begun to consume. From the 112 patients that had eaten sharon fruit before, 19 (17%) reported reactions to it. The 17% found in this study might be an underestimation, since the patients were evaluated only by questionnaires and might not be familiar with an uncommon fruit like this.

Recently, we reported similar findings for an even less common fruit, i.e. jack fruit [28]. From the regulatory point of view, it is doubtful whether sharon fruit or jack fruit would now still be allowed to enter the market. A recent report [42] on a tropical nut, the nangai nut, suggests that this novel food should perhaps have been refused a marketing authorization on the basis of allergenicity following the EC guidelines on novel foods. Whether allergenicity of a novel food is reason enough to deprive all consumers from access to such foods remains an interesting debate.

In summary, we have demonstrated that allergy to sharon fruit is a new member of birch-pollen-related fruit allergy. Bet v 1 plays an important role in serological cross-reactivity, as demonstrated in contrast to previous studies. The clinical relevance of the different allergens involved need further studies.

Acknowledgement

This study was supported by a grant from the EC: SAFE QLK1-CT-2000-01394.

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