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# Component-resolved diagnosis of plant food allergy by SPT

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## KEY WORDS

Food allergy, fruits, vegetables, SPT, allergens

## SUMMARY

**Background:** Fruits and vegetables may contain both labile and stable allergens. The former induce only OAS, whereas stable allergens may induce systemic reactions. Component-resolved diagnosis (CRD) of allergy to plant foods is therefore essential for the clinical management of allergic patients. **Methods:** 80 adults allergic to plant foods underwent SPT with purified natural date palm profilin (Pho d 2), purified Mal d 1, a peach extract containing uniquely LTP, and with a kiwi extract containing uniquely stable allergens. **Results:** 58 (72%) patients were monosensitized: 24 to Mal d 1, 24 to profilin, 7 to LTP, and 3 to kiwi. 22 patients were multi-sensitized: 14 to Mal d 1 and profilin, 2 to Mal d 1 and kiwi, 1 to LTP and profilin, 3 to LTP and Mal d 1, and 2 to LTP, Mal d 1 and profilin. Mal d 1 and LTP sensitisation were associated with apple and peach allergy, respectively, whereas profilin sensitisation was associated with allergy to melon, watermelon, banana, tomato and citrus fruits. 18/21 kiwi-allergic patients were sensitised to one of the cross-reacting allergens, but 2/18 reacted to kiwi-specific allergens as well. **Conclusions:** In patients with allergy to plant-derived foods CRD can be performed by SPT with purified allergen proteins. In the future, the availability of a larger number of purified natural or recombinant allergens for SPT will represent a simple means to classify food-allergic patients properly on the first visit.

## Introduction

Plant-derived foods represent by far the most frequent cause of food allergy in adults. One of the main features of fruits and vegetables as food allergens is that they may contain both labile and stable allergen proteins. This fact strongly influences the clinical presentation of allergy to a certain food as well as the risk associated with re-exposure to the offending food and/or to potentially cross-reactive ones. In effect, in virtually all sensitised patients pepsin-sensitive proteins induce only mild local symptoms (i.e., oral allergy syndrome, OAS), whereas more stable aller-

gens reach the gastro-intestinal tract in a biologically active form and may induce potentially severe systemic symptoms (1-4). For the clinician this scenario is further complicated by the fact that, due to unclear reasons, subjects sensitised to stable allergens may have both mild (OAS) and/or systemic symptoms (5). The precise detection of the sensitizing allergen protein(s) in patients allergic to plant-derived foods is therefore extremely important to give patients correct advice about their clinical condition and about the necessity to exclude or not certain foods from their diet.

Fruits and vegetables contain 3 main, highly cross-reacting allergens two of which, namely profilin (6-9) and pro-

teins homologous to Bet v 1 (7,10,11), are extremely pepsin-sensitive whereas one, the non-specific lipid transfer protein (LTP), is very heat- and pepsin-stable (12-14). Up to now, in normal clinical settings the detection of sensitisation to these allergens has been based on indirect parameters such as the presence/absence of birch pollen hypersensitivity and/or the presence of hypersensitivity to many botanically unrelated pollens, suggestive of profilin hypersensitivity (these aspects have been reviewed in [15]). Another plant derived food that is causing an increasing number of allergies is kiwi. Again, prognosis of kiwi allergy is variable as allergic patients can be birch-pollen sensitised subjects (due to sensitisation to Bet v 1-homologous proteins) (16), profilin reactors (17), sensitised to kiwi-specific allergens (e.g. Act c 1) (18), or may have a latex-fruit syndrome (19); the 2 latter categories have theoretically a more risky clinical condition.

In recent years an increasing number of allergenic molecules have been sequenced and cloned, and are now available for routine in-vitro diagnostics; however, at the present most of these proteins are airborne allergens and most of the few available food allergens are of animal origin. The possibility to detect the sensitising allergen on the first visit in patients with a history of allergy to fruits and/or vegetable would represent a real step forward in the clinical practice both in terms of clinical care and of reduction of costs. The present study evaluated the effectiveness of component resolved diagnosis of plant-food allergy by means of SPT with extracts of plant-derived foods containing one single allergen protein due either to the loss of labile allergens during the preparation process, or to a proper purification procedure of the relevant protein.

## Methods

### *Patients*

80 consecutive patients (M/F 32/48; mean age 36 [SD 14.7] years, range 9-70 years) with a clinical history of allergy to plant derived foods confirmed by positive SPT with fresh offending food or with commercial food extract (1/20 wt/vol; ALK-Abello, Spain) seen at the allergy centre of the Clinica San Carlo were studied. A history of oral allergy syndrome (defined as itching of the oral mucosa and lips with or without angioedema immediately after eating specific foods), of urticaria with or without angioedema, and/or of severe gastrointestinal disorders fol-

lowing the ingestion of specific plant-derived foods was considered as compatible with food allergy and hence used as an inclusion criterion.

### *Skin tests*

SPT with commercial extracts of pollens present in this geographical area, including grass, mugwort, ragweed, pellitory, plantain, birch, olive (all 50000 SBU/ml; Allergopharma, Reinbek, Germany) and cypress (30 HEP, ALK-Abello, Spain) were carried out in all patients. Further, all patients underwent SPT with purified natural date palm profilin (Pho d 2; 50 µg/ml; Alk Abello, Madrid, Spain; see beyond), with an apple extract containing uniquely Mal d 1 (2 µg/ml; ALK-Abello; see beyond), with a commercial peach extract containing uniquely lipid transfer protein (LTP 30 µg/ml; ALK-Abello)(15, 16), with a kiwi extract (5% w/v; ALK-Abello), and with a commercial natural rubber latex extract (500 µg protein/ml; ALK-Abello).

In a preliminary study on 36 patients with kiwi allergy positive on SPT with fresh kiwi by prick-prick technique the SPT with this commercial kiwi extract scored positive only in 8 cases, all without pollen-food allergy syndrome (5 patients were monosensitized to kiwi, 2 has latex-fruit allergy syndrome, and 1 patient was sensitized to LTP), whereas it did not induce any skin reaction in 28 patients with pollen-food allergy syndrome many of whom showing high levels of kiwi-specific IgE on CAP. It was therefore concluded that, similarly to apple extracts for SPT (10,20), this kiwi extract lacks both the allergen homologous to Bet v 1 and profilin, and contains uniquely stable allergens.

All SPT were performed using disposable 1 mm tip lancets (ALK-Abello). Readings were taken at 15 min, and a mean wheal diameter of 3 mm or more was considered positive (21). SPT with histamine 10 mg/ml and saline were carried out as positive and negative control, respectively.

### *Preparation of apple and Pho d 2 extracts*

Lyophilized apple peels were extracted for 90 minutes at 4° with 0.1 mol/L sodium carbonate/bicarbonate, 0.1 mol/L Na Cl, pH 9.4. Extracts were centrifuged (1000 rpm, 30min, 4°C) and 50% glycerol was added. Apple extract contained 2 µg/ml of Mal d 1 as determined by ELISA, Mal d 4 < 0.1 µg/ml, Mal d 3 < 0.05 µg/ml. Natural profilin Pho d 2 was purified from date palm ex-

tract by affinity chromatography with a poly-L-proline-Sepharose (22); purity was checked by SDS PAGE, mass spectrometry and amino acid analysis. The concentration of Pho d 2 in the extract was 50 µg/ml

### Statistics

Associations were assessed by the chi-square test with Yates' correction. P values < 0.05 were considered statistically significant.

## Results

### Clinical presentation of food allergy

All patients reported oral allergy syndrome as the only symptom of food allergy except 4 who also had urticaria (2 cases), asthma, rhinitis and gastro enteric symptoms (1 case) and gastric pain (1 case).

### SPT with food allergens and clinical associations

All 80 patients scored positive with at least one out of 4 allergens tested (namely, profilin, Mal d 1, LTP, and kiwi-specific allergens). Results are summarized in table 1. Fifty-eight/80 (72%) patients turned out to be monosensitized (24 to profilin, 24 to Mal d 1, 7 to LTP, and 3 to kiwi), whereas 22 were sensitised to > 1 allergen (14 Mal d 1 + profilin; 3 Mal d 1 + LTP; 2 Mal d 1 + Kiwi; 2 Mal d 1 + profilin + LTP; and 1 profilin + LTP). No patient scored positive on SPT with latex extract.

In patients sensitised to the 3 cross-reacting allergens (Mal d 1, profilin, and LTP) the pattern of offending

foods changed with the sensitising allergen (Tab. 2). Apple allergy was significantly associated with sensitisation to Mal d 1; 30/45 (67%) Mal d 1-hypersensitive subjects had apple allergy (p < 0.05). Peach allergy was associated with sensitisation to lipid transfer protein; 12/13 (92%) LTP-hypersensitive patients were allergic to this fruit (p < 0.05). Finally allergy to melon, watermelon, citrus fruit, banana, and tomato was significantly associated with profilin sensitisation; of 41 profilin-hypersensitive patients 24 (58%), 14(34%), 19 (46%), 10 (24%), and 9 (22%) were allergic to melon, watermelon, tomato, banana, and citrus fruits, respectively (p < 0.05-0.001).

The 3 patients reporting oral allergy syndrome plus food-induced urticaria (n = 2) or plus asthma, rhinitis, and gastro enteric symptoms (n = 1) were sensitised to LTP. The patient reporting OAS and gastric pain was monosensitized to kiwi. Clinically, patients sensitised to kiwi-specific proteins reported a much more severe oral symptoms (frequently associated with oedema of the lips, tongue, and pharynx and with tightness of throat) than kiwi-allergic patients sensitised to Mal d 1 and/or profilin.

### Association with pollen hypersensitivity

Hypersensitivity to seasonal airborne allergens tested is shown in table 3. Not surprisingly, all Mal d 1-sensitized patients were allergic to birch pollen, and profilin-hypersensitive patients were sensitised to most pollens, with the partial exception of Parietaria and cypress. Most LTP-allergic patients were sensitised to grass pollen. In contrast, patients monosensitized to kiwi were not sensitised to seasonal allergens with the exception of one subject sensitised to grass pollen.

**Table 1** - Skin reactivity and clinical symptoms in 80 consecutive patients with plant-food allergy

Skin reactivity	No.	OAS	OAS + urticaria	OAS + RAG	OAS + G
Mal d 1 only	24	24			
Profilin only	24	24			
LTP only	7	6		1	
Mal d 1 + Profilin	14	14			
Profilin + LTP	1		1		
Mal d 1 + Profilin + LTP	2	2			
Mal d 1 + LTP	3	2	1		
Mal d 1 + Kiwi	2	2			
Kiwi only	3	2			1

R: rhinitis; A: asthma; G: gastrointestinal

**Table 2** - Offending foods and statistical associations in 80 patients allergic to plant-derived foods sensitised to different allergen proteins

No. Patients	M	P	L	MP	PL	MPL	ML	MK	K	p		
										M	P	L
Apple	16	9	2	10	1	1	3			<0.05	NS	NS
Pear	8	4	0	3		1	1			NS	NS	NS
Peach	12	12	7	7	1	1	2			NS	NS	<0.05
Cherry	12	7	1	2		1	3			NS	NS	NS
Plum	6	3	0	3		1	0			NS	NS	NS
Apricot	7	6	0	6		0	2			NS	NS	NS
Medlar	2	0	0	1		0	0			NS	NS	NS
Almond	4	2	1	0		0	0			NS	NS	NS
Hazelnut	10	8	3	6		1	1			NS	NS	NS
Walnut	6	6	2	1		1	0			NS	NS	NS
Peanut		2	2	0		0	0			NS	NS	NS
Melon	2	17	0	6		1	0			NS	<0.001	NS
Watermelon	1	10	0	3		1	0			NS	<0.01	NS
Orange/tang	1	7	0	1		1	0			NS	<0.05	NS
Grapes	0	2	0	1		1	0			NS	NS	NS
Pineapple	1	4	1	2		0	0			NS	NS	NS
Banana	0	8	0	2		0	0			NS	<0.01	NS
Carrot	7	3	0	3		0	0			NS	NS	NS
Celery	4	2	0	1		0	0			NS	NS	NS
Fennel	4	2	1	2		0	1			NS	NS	NS
Tomato	1	12	1	5		2	0			NS	<0.001	NS
Eggplant	1	2	1	0		0	0			NS	NS	NS
Lettuce	0	1	0	0		0	0			NS	NS	NS
Kiwi	4	4	2	5		0	1	2	3	NS	NS	NS
Strawberry	2	1	1	3		0	0			NS	NS	NS
Zucchini	0	1	0	0		0	0			NS	NS	NS
Persimmon	0	1	0	0		1	0			NS	NS	NS
Coconut	0	1	1	0		0	0			NS	NS	NS

M: Mal d 1; P: Profilin; L: Lipid transfer protein; K: Kiwi specific allergens

## Discussion

The present study highlights the importance of being able to carry out a component-resolved diagnosis in-vivo when the patient with plant food allergy is seen for the first time in the clinic. In keeping with previous studies, all patients sensitised to labile allergens had oral allergy syndrome as the only clinical expression of their food allergy (10,23); however, also most of the patients sensitised to stable vegetable food allergens in this study reported oral allergy syndrome. Further, 22/80 (28%) patients were multi-sensitised to vegetable food allergens, and 8 of

these subjects reacted to both labile (Mal d 1 and/or profilin) and stable (LTP and/or kiwi) allergen proteins. In these patients component-resolved diagnosis has been essential to give the correct advice about the possibility to maintain (e.g., in subjects sensitised to labile proteins) or the necessity to change dietary habits (e.g. avoidance of kiwi fruit in specifically sensitised subjects; avoidance of whole fresh Rosaceae with or without tree nuts, as well as avoidance of commercial Rosaceae fruit juices in LTP-allergic patients), or how to reduce allergenicity of potentially offending foods (e.g. to try peeled Rosaceae fruits in LTP-allergic subjects [24]; to eat fruit salads and drink

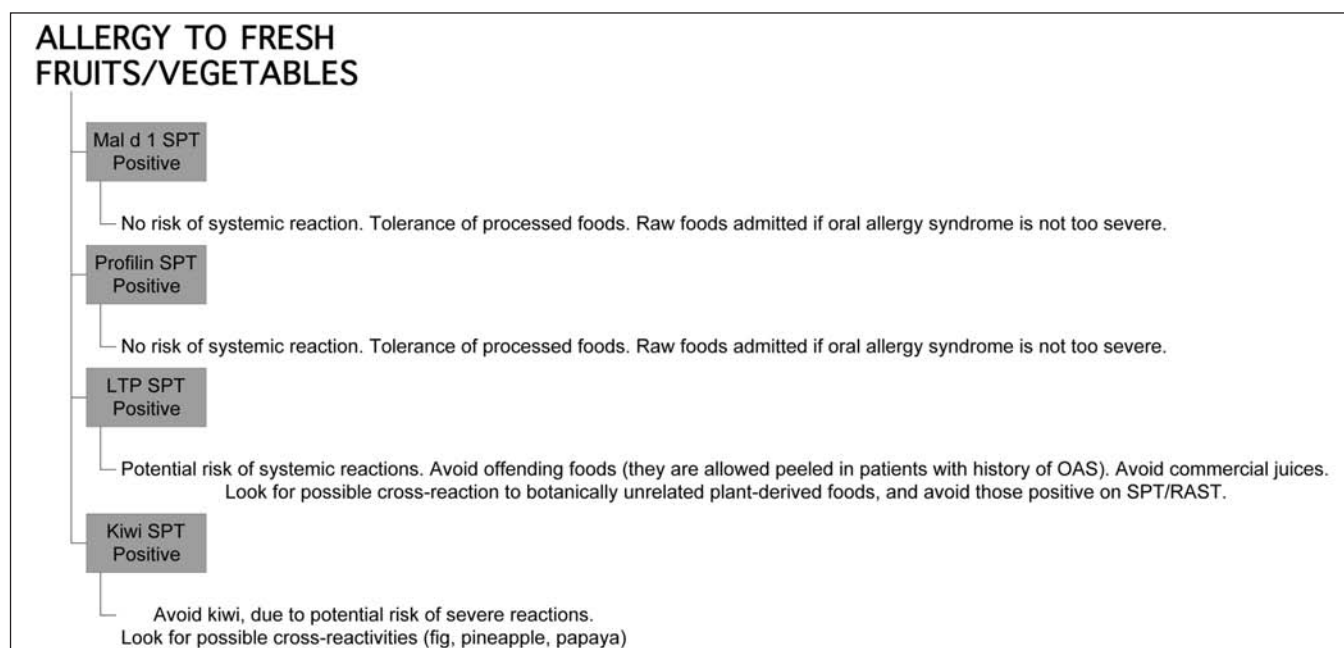
**Table 3** - Sensitivity to pollens in 80 patients allergic to plant foods

Sensitivity	Grass	Mugwort	Ragweed	Pellitory	Plantain	Birch	Olive	Cypress
Mal d 1 only (n=24)	11 (46%)	8 (33%)	12 (50%)	4 (17%)	5 (21%)	24 (100%)	9 (38%)	10 (42%)
Profilin only (n= 24)	24 (100%)	22 (92%)	23 (96%)	10 (42%)	21 (88%)	23 (96%)	23 (96%)	10 (42%)
LTP only (n= 7)	6 (86%)	1 (14%)	3 (43%)	0 (0%)	0 (0%)	1 (14%)	0 (0%)	0 (0%)
Mal d 1+Profilin (n=14)	14 (100%)	13 (93%)	13 (93%)	9 (64%)	14 (100%)	14 (100%)	13 (93%)	8 (57%)
Profilin + LTP (n=1)	1 (100%)	1 (100%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	1 (100%)	0 (0%)
Mal d 1 + profilin + LTP (n=2)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)
Mal d 1 + LTP (n= 3)	3 (100%)	0 (0%)	1 (33%)	1 (33%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)
Mal d 1 + Kiwi (n= 2)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)	0 (0%)
Kiwi (n=3)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total Mal d 1 (n= 45)	32 (71%)	24 (53%)	30 (67%)	17 (38%)	23 (51%)	45 (100%)	26 (58%)	18 (40%)
Total Profilin (n= 41)	41 (100%)	38 (93%)	39 (95%)	21 (51%)	38 (93%)	40 (98%)	39 (95%)	18 (44%)
Total LTP (n=13)	12 (92%)	4 (31%)	7 (54%)	3 (23%)	3 (23%)	7 (54%)	3 (23%)	0 (0%)

commercial fruit juices in patients sensitised to labile allergens [15]). The validity of the diagnostic means used in this study is further, indirectly confirmed by the observed associations. Apple has been known as the clinical marker of birch/food allergy syndrome for a long time (25); similarly, peach allergy is a “trademark” of LTP hypersensitivation

(26, 27), and the association between profilin sensitisation and allergy to melon, watermelon, citrus fruit, banana, and tomato is in keeping with previous studies (28, 29).

The panel of plant-food allergens used in this study was obviously incomplete as relevant cross-reacting allergens

**Figure 1** - Allergy to fresh fruits/vegetables

such as the seed storage proteins, including 2S-albumins, vicilins, and legumins, were missing. Nonetheless, no patients out of the 80 consecutive ones included in the present study reported a history of systemic reactions to tree nuts and/or seeds, which suggests that (at least in this area) allergy to seed storage proteins is much less frequent than allergy to Bet v 1-homologue proteins, profilins, LTP or kiwi. Hopefully, these purified proteins as well will be available for in-vivo testing in the future. A summary of the clinical use of these 4 allergens is suggested in figure 1.

One further aspect that is worth discussing is the advantage of performing SPT with purified proteins rather than carrying out a molecular analysis by in-vitro tests. Presently, both the immunoCAP (Phadia, Uppsala, Sweden) and the protein micro-array ISAC (VBC Genomics- Phadia) include recombinant profilin, Bet v 1-homologous proteins and LTP (Pru p 3 and others) in their panels. The latter also includes kiwi-specific allergens. However, both assays are more expensive than a simple SPT. Particularly, the micro-array is a "take it or leave it" test in which one is forced to measure IgE specific for > 90 allergen proteins, even if the diagnostic question deals with 3-4 allergens; the immunoCAP is still unable to produce a differential diagnosis between primary or secondary kiwi allergy. Finally, the results that both assay produce are not readily available. The immunoblot analysis is another common means to investigate allergenic proteins in-vitro, but it is still not available in most clinical settings.

In conclusion, in recent years molecular biology techniques have much improved the diagnosis of allergy, and several allergen proteins are already available for in-vitro assays, although the number of food allergens still remains limited. Purified food allergen proteins are being (slowly) introduced also for in-vivo testing and this will enormously simplify doctors' work and improve patients' care.

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