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Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen

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Clinical and Experimental Allergy

Summary

Background Profilin, a pan-allergen present in all eukaryotic cells, is one of the main causes of cross-sensitization between pollen and plant-derived foods, but its clinical relevance as a food allergen is still debated.

Objective To investigate the prevalence of profilin sensitization in a pollen-allergic population and its clinical relevance as a food allergen.

Methods Two hundred consecutive patients with pollen allergy underwent skin prick tests (SPT) with purified natural date palm profilin (Pho d 2; 50 µg/mL; ALK-Abello, Madrid, Spain). Those reporting adverse reactions to foods (confirmed by SPT with either commercial food extracts or fresh foods) underwent SPT with an apple extract containing uniquely Mal d 1 (2 µg/mL; ALK-Abello), and with a commercial peach extract containing uniquely lipid transfer protein (LTP 30 µg/mL; ALK-Abello).

Results Sixty patients (30%) showed skin reactivity to date palm profilin, Pho d 2. All were sensitized to grass pollen, and most of them reacted to birch, mugwort, ragweed and plantain pollen as well. SPT with pellitory and cypress scored negative in a high proportion of profilin reactors [26/60 (43%) and 33/60 (55%), respectively]. More than one half (34/60 [57%]) of profilin reactors had food allergy; 21 of these were monosensitized to profilin, 11 were sensitized to both profilin and Bet v 1 homologous protein, one to both profilin and LTP, and one to all the three allergens. The large majority of profilin-allergic patients reported oral allergy syndrome as the only food-induced symptom and were able to tolerate the offending foods if they were cooked or otherwise processed. Twenty-eight of 34 reported reactivity to two or more plant-derived foods. Rosaceae, tree nuts, melon and watermelon, tomato, pineapple, citrus fruits and banana were the more frequently offending foods.

Conclusion Profilin should be considered a clinically relevant food allergen. Allergy to melon, watermelon, tomato, banana, pineapple and orange may be considered as a marker of profilin hypersensitivity. This study underlines the clinical importance of being able to diagnose hypersensitivity to single food allergenic proteins by SPT, particularly when the relevant food allergen sources contain several allergens that show different chemical/physical features and, hence, completely different risk profiles.

Keywords component-resolved diagnosis, food allergy, profilin, skin prick tests

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Introduction

Profilin, a highly conserved 12–15 kDa protein present in all eukaryotic cells, is one of the main causes of cross-sensitization between pollen and plant-derived foods [1–4]. It is considered a minor pollen allergen, as it has been reported that only 10–20% of patients with pollen allergy are sensitized to this protein [2, 3, 5]. Although the

cross-sensitization to vegetable foods among profilin reactors has been known for a long time, the clinical relevance of profilin as a food allergen is still debated. There have been studies claiming that this protein has little or no clinical relevance [5–7] along with other works concluding that, at least in a proportion of cases, it is a clinically relevant food allergen [8, 9]. Such discrepancies are possibly due to population selection criteria. In effect,

where patients were selected on the basis of serological IgE reactivity the clinical relevance of the allergen appeared to be rather poor, whereas where study populations were selected on the basis of a history of food allergy the allergen turned out to be clinically relevant. Systematic studies carried out on large populations of patients with pollen allergy aiming to assess both the prevalence and the clinical relevance of profilin hypersensitivity in food allergy are still missing, probably as a consequence of the lack of a cheap and easy routine means to detect sensitized patients. To know the prevalence and clinical relevance of profilin as a food allergen would be a relevant information for the clinicians that have to manage patients with food allergy. Recently, a purified natural profilin for skin prick tests (SPTs) has become commercially available in some European countries. This extract has been employed in the present study to investigate the prevalence of hypersensitivity of this protein in patients with pollen allergy, and to assess the clinical relevance of profilin as a food allergen.

Materials and methods

Patients

Consecutive adult patients with a clinical history of suspected pollen allergy recently seen at the allergy centre of the Clinica San Carlo, Paderno Dugnano, Italy, were considered for this prospective study with the aim to eventually include a total of 200 subjects with clinically defined pollinosis. Based on the mean pollen seasons of this geographic area [10, 11], a history of rhino-conjunctivitis with or without asthma from end February to mid May, from end April to mid July and from mid August to the end of September was considered as possibly associated with respiratory allergy to birch and/or cypress, grass and/or pellitory and ragweed and/or mugwort, respectively.

All subjects underwent a thorough interview in order to ascertain possible food allergy. A clear-cut history of oral allergy syndrome (defined as immediate itching of the oral mucosa and lips), of urticaria (with or without angioedema) and/or of severe gastrointestinal disorders following the ingestion of specific plant-derived foods was considered compatible with food allergy.

Skin tests

All study patients underwent SPT with commercial extracts of the pollens present in this geographical area, including grass, mugwort, ragweed, pellitory, plantain, birch, olive (all 50 000 SBU/mL; Allergopharma, Reinbek, Germany) and cypress (ALK-Abello, Madrid, Spain). SPT with purified natural date palm profilin (Pho d 2; 50 µg/mL; Alk Abello, Madrid, Spain), with an apple extract contain-

ing uniquely Mal d 1 (2 µg/mL; ALK-Abello) [11] and with a commercial peach extract containing uniquely lipid transfer protein (LTP 30 µg/mL; ALK-Abello) [12, 13] were performed in all patients as well. Further, in patients reporting adverse reactions following the ingestion of plant foods, SPT with commercial extracts of tomato, hazelnut, carrot, celery, kiwi and peanut (ALK-Abello) and/or with fresh foods (in the case of allergy to all foods) were performed in order to confirm sensitization.

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 [14]. SPT with histamine (10 mg/mL) and saline were carried out as positive and negative control, respectively.

Statistics

Proportions were compared by χ^2 test with Yates' correction. Probability values < 0.05 were considered significant.

Results

Study population and prevalence of profilin sensitization

Two hundred and fourteen consecutive subjects with a history of suspected pollen allergy underwent the diagnostic procedure in order to reach the scheduled number of 200 allergic patients. In fact, 14 subjects were finally diagnosed as not suffering from pollen allergy: two were sensitized to mites, two to *Alternaria* and 10 were non-allergic and had experienced long-lasting rhinitis; in all these 14 subjects profilin, Mal d 1, and LTP scored negative on SPT.

The clinical features of the study population ($n = 200$) are shown in Table 1. The mean age was 33.3 years (range 4–73 years) and sex distribution was F/M: 107/93.

Sixty (30%) patients showed skin reactivity to date palm profilin, Pho d 2. Patients sensitized and not sensitized to profilin did not differ in mean age (33.3 vs. 33.4 years, respectively; $P = \text{NS}$), nor in the slight female prevalence (M/F: 27/33 vs. 66/74, respectively; $P = \text{NS}$).

Skin prick tests with pollen extracts in profilin reactors

All 60 profilin reactors scored positive on SPT with grass pollen commercial extract. Birch, mugwort, ragweed, olive and plantain pollen extracts scored negative in a minority of profilin reactors ($n = 1, 3, 3, 4$ and 6 , respectively), whereas, interestingly, pellitory and cypress extracts scored negative in a high proportion of cases [26/60 (43%) and 33/60 (55%), respectively]. Pollen reactivity in patients sensitized and not sensitized to profilin is shown in Table 2. Not surprisingly, profilin reactors showed a

Table 1. Clinical features of the study population

	Total population	Profilin reactors	Non-profilin reactors	P
No.	200	60	140	
Mean age (range) (years)	33.3 (4–73)	33.3 (8–63)	33.4 (4–73)	NS
M/F	107/93	27/33	66/74	NS
No. reporting food allergy	61 (30%)	34 (57%)	27 (19%)	< 0.001

Table 2. Pollen sensitization in patients sensitized and non-sensitized to profilin

Pollen	Profilin-positive (n = 60)	Profilin-negative (n = 140)	P
Grass	60 (100%)	81 (58%)	< 0.001
Mugwort	57 (95%)	48 (34%)	< 0.001
Ragweed	57 (95%)	104 (74%)	< 0.01
Pellitory	34 (57%)	17 (12%)	< 0.001
Plantain	56 (93%)	19 (14%)	< 0.001
Birch	59 (98%)	54 (39%)	< 0.001
Olive	56 (93%)	28 (20%)	< 0.001
Cypress	27 (45%)	29 (21%)	< 0.001

significantly higher prevalence of positive SPT with all pollens tested.

Food allergy and association with profilin hypersensitivity

A total of 61 patients reported allergies to plant-derived foods that were confirmed by SPT. On SPT with the three food allergen extracts studied and with commercial food extracts, 27 patients turned out to be sensitized to plant food allergens other than profilin [i.e. Bet v 1-like allergens and/or LTP in 26 cases; actinidin (kiwi) in one case], whereas 34/61 (56%) were sensitized to profilin. Of these 34, 21 were probably monosensitized to profilin, 11 were sensitized to both profilin and Bet v 1 homologous protein, one to both profilin and LTP and one to all the three allergens. More than one-half of profilin reactors reported food-induced symptoms (34/60, 57%). Profilin sensitization was significantly associated with food allergy ($P < 0.001$; Table 1).

The offending foods reported by profilin-sensitized patients are shown in Table 3. All profilin-allergic patients reported oral allergy syndrome as the only food-induced symptom with the exception of one case of melon- and tomato-induced urticaria, one case of tomato-induced gastric pain, and one case of peach-associated exercise-induced urticaria/angioedema in the patient sensitized to both profilin and LTP. Further, all patients but those sensitized to LTP reported that the offending foods were tolerated if they were cooked or otherwise processed (e.g. commercial juices). Only six profilin reactors reported intolerance to a single food (two tomato, two melon, one hazelnut, one orange); the remaining 28 reported reactiv-

Table 3. Offending foods in patients sensitized and non-sensitized to profilin

Food	Profilin-positive (n = 34)	Profilin-negative (n = 27)	P
Melon	19 (56%)	1 (4%)	< 0.001
Peach	18 (53%)	14 (52%)	NS
Apple	17 (50%)	18 (67%)	NS
Tomato	17 (50%)	2 (7%)	< 0.001
Hazelnut	13 (38%)	10 (37%)	NS
Apricot	11 (32%)	8 (30%)	NS
Watermelon	11 (32%)	1 (4%)	< 0.001
Cherry	8 (24%)	12 (44%)	NS
Banana	8 (24%)	0 (0%)	< 0.05
Walnut	7 (21%)	6 (22%)	NS
Kiwi	7 (21%)	7 (26%)	NS
Pineapple	6 (18%)	2 (7%)	NS
Orange/tangerine	6 (18%)	1 (4%)	NS
Carrot	4 (12%)	6 (22%)	NS
Plum	4 (12%)	6 (22%)	NS
Strawberry	4 (12%)	2 (7%)	NS
Pear	4 (12%)	7 (26%)	NS
Fennel	4 (12%)	6 (22%)	NS
Celery	3 (9%)	3 (11%)	NS
Grapes	3 (9%)	0 (0%)	NS
Peanut	2 (6%)	1 (4%)	NS
Eggplant	2 (6%)	0 (0%)	NS
Coconut	1 (3%)	1 (4%)	NS
Medlar	1 (3%)	2 (7%)	NS
Lettuce	1 (3%)	0 (0%)	NS
Fig	1 (3%)	1 (4%)	NS
Zucchini	1 (3%)	0 (0%)	NS
Almond	1 (3%)	5 (19%)	NS
Persimmon	1 (3%)	0 (0%)	NS

ity to two or more plant-derived foods. Interestingly, along with Rosaceae and tree nuts, some foods that have been rarely associated with allergy to birch pollen or to LTP, such as the gourd family, tomato, pineapple, citrus fruits and banana, were among those most frequently reported by profilin reactors. The association between allergy to melon, watermelon, tomato and banana, and profilin sensitization was statistically significant. Further, profilin reactors showed a higher prevalence of allergy to pineapple and citrus fruits although in these cases, due to the low numbers, the difference did not reach statistical significance. Of 60 profilin reactors, 26 (43%) did not report any food-induced adverse reaction.

Discussion

We enrolled a large number of consecutive patients in this prospective study in order to get a study group that was representative of the general population of subjects with pollen allergy seen in this area. The prevalence of profilin sensitization in this study was 30%, a proportion that is higher than those reported in previous studies [1, 5]. This difference might be due to geographical factors. Most data of the prevalence of profilin sensitization come from areas where birch pollen allergy is highly represented as shown by the fact that profilin was first detected as an allergen in birch pollen [3], and the first studies were performed in birch pollen-allergic populations [2]. It is possible that in areas where other pollens prevail, such as northern Italy where grass and ragweed are the most relevant allergenic sources, patients may show a higher propensity to get sensitized to profilin. In effect, it has been suggested that profilin, which is a minor allergen in birch pollen might represent an intermediate or major allergen in patients sensitized to grasses or weeds [3]. In the clinical practice, adult patients polysensitized to different seasonal allergen sources (which suggests co-recognition of shared allergen proteins) are very commonly observed in this allergy centre. Further, it is tempting to speculate that the use of a natural profilin leads to an improved sensitivity in the detection of sensitization to this allergen, as a lower IgE-binding activity of rBet v 2 compared with its natural counterpart has been observed [7], pointing to possible differences between natural and recombinant allergens.

Profilin has been considered as a plant pan-allergen for a long time; however, in this study profilin sensitization was frequently found in the absence of IgE reactivity to cypress and pellitory pollen, suggesting that profilins from these two plants are either present in relatively low amounts, show a different *in vivo* reactivity, or show some epitopic difference from profilins from all other plants. The poor cross-reactivity with *Parietaria* profilin confirms previous observations [15], whereas the finding with *Cupressus* is a novel one and should deserve to be investigated further.

More than one half of profilin-sensitized patients included in this study showed clinically relevant cross-sensitization to plant-derived foods, suggesting that profilin should be considered a clinically relevant food allergen. This conclusion, which contradicts that of other studies [5, 7], is supported also by the fact that clinical food allergy was present in patients probably mono-sensitized to profilin. The large majority of profilin reactors with food allergy reported only the typical oral allergy syndrome following the ingestion of the offending foods, which is in keeping with the known pepsin-sensitivity of this allergen [16]. It is particularly interesting to observe that the list of the most frequently offending foods included some fruits and vegetables which have

been rarely associated to sensitization to other plant food allergens (i.e. proteins homologous to Bet v 1 and LTP) such as melon and watermelon, tomato, banana, pineapple and orange. These findings are in agreement with previous observations [16–20] and confirm that allergy to these fruits may be considered as a marker of profilin hypersensitivity [9]. Like in previous studies [21–23], apple, peach and other fruits belonging to Rosaceae family were also among the most frequently offending foods in profilin-allergic patients. The low prevalence of clinical allergy to plant-derived foods that have been associated with profilin reactivity (e.g. persimmon [24]) is probably due to the fact that such foods are present in the market for a short period, and to the low frequency by which these foods are consumed by the general population. Although this study assessed sensitization to Mal d 1 and LTP, other allergens involved in plant-food allergy, such as thaumatin-like protein (TLP) in fruits (apple, grapes, bell pepper and kiwi) and 2S albumins in nuts, were not investigated. Nonetheless, it seems rather unlikely that this may have somehow biased the results. Sensitization to TLP is rare and its clinical significance is still debated (reviewed in [13]), whereas sensitization to 2S albumins seems rather unlikely in this population, as these allergens are extremely stable and are generally associated with severe systemic symptoms [25] whereas virtually all nut-allergic patients in this study had only oral allergy syndrome.

In conclusion, this study highlights the clinical importance of being able to diagnose hypersensitivity to single food allergenic proteins immediately in the office by SPT; this is particularly useful for the clinical allergologist if the relevant food allergen sources contain several allergenic proteins that show different chemical/physical features and, hence, completely different risk profiles. The possibility to carry out SPT with single protein allergens rather than with mixtures might become an invaluable diagnostic tool for the clinicians in the future.

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