

***Parietaria* Profilin Shows Only Limited Cross-Reactivity with Birch and Grass Profilins**

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Key Words

Allergens · Birch · Cross-reactivity · Grass · *Parietaria* · Pollen · Profilin

Abstract

Background: In patients showing multiple reactivity to seasonal airborne allergens, a skin prick test (SPT) with *Parietaria* pollen scores frequently negative, suggesting that profilin from *Parietaria* might not share IgE-binding epitopes with profilin from botanically unrelated airborne allergens. This study investigated the immunologic cross-reactivity between profilins from *Parietaria*, grass and birch pollen. **Methods:** 36 patients hypersensitive to birch profilin, Bet v 2, underwent SPT with *Parietaria*, and IgE to both whole *Parietaria* pollen and *Phleum* profilin were sought in their sera by ELISA. In ELISA inhibition studies, IgE reactivity to *Phleum* profilin was measured before and after absorption of sera from *Parietaria* reactors with both whole *Parietaria* pollen extract, and Par j 1/Par j 2 fraction. Further, their IgE reactivity to whole *Parietaria* pollen was measured before and after absorption with the Par j 1/Par j 2 fraction. **Results:** All sera showed IgE reactivity to *Phleum* profilin. Only 14/36 (39%) patients had a positive SPT to *Parietaria* and 17/36 (47%) showed IgE to *Parietaria*. Absorption of sera from *Parietaria* reactors with whole *Parietaria* extract caused a marked reduction in IgE reactivity to grass profilin; in

contrast, absorption of sera with the Par j 1/Par j 2 fraction did not exert any inhibitory effect on IgE reactivity to grass profilin. Absorption of sera with the Par j 1/Par j 2 fraction markedly reduced IgE reactivity to whole *Parietaria* extract in 8/9 cases. **Conclusion:** Less than 50% of patients sensitized to birch and grass profilin cross-react to *Parietaria* profilin. In most cases, cross-reactors are hypersensitive to major, specific *Parietaria* allergens as well. This findings may be of practical relevance when the prescription of specific immunotherapy is considered.

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Introduction

Profilin is a 12- to 15-kD monomeric actin-binding protein present in all eukaryotic cells. It was first described as a minor allergen in birch pollen [1], but on the basis of subsequent studies demonstrating its wide distribution and cross-reactivity profilin is presently considered a ubiquitous plant pan-allergen [2, 3] and one of the main causes of cross-sensitization between pollen and plant-derived foods. Patients sensitized to this protein typically show skin and serological reactivity to an array of seasonal airborne allergens. Surprisingly, skin prick test (SPT) to *Parietaria officinalis* pollen extract scores frequently negative in patients showing multiple sensitivities

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1018–2438/04/1332–0121\$21.00/0

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to seasonal allergens. Since the presence of profilin in *Parietaria* pollen has been established [4], this observation suggests that *Parietaria* profilin might not share IgE-binding epitopes with profilin from other seasonal airborne allergens. This study aimed to investigate the immunologic cross-reactivity between profilins from *Parietaria*, grass and birch pollen.

Patients and Methods

Patients

The study was carried out on 36 adult patients (12 males/24 females; age 16–44 years) referred to the allergy unit of the hospital of Bollate, Italy, for routine care of their airborne allergy. These patients were selected from a large number of allergic patients on the basis of (i) a history of oral allergy syndrome following the ingestion of different plant-derived foods; (ii) positive SPT to a series of airborne allergens including grass, mugwort, ragweed, plantain, birch, hazel, olive and cypress, and (iii) IgE reactivity to recombinant birch profilin, rBet v 2, by CAP system (Pharmacia, Uppsala, Sweden).

Skin Tests

Patients underwent SPT to a commercial extract of *P. officinalis* pollen (Allergopharma, Reinbeck, Germany; 50,000 standardized biological units/ml). Skin tests were carried out on the volar side of the forearm using sterile 1-mm-tip lancets (Dome-Hollister/Stier). Readings were taken after 15 min. Reactions were expressed as mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing it by 2). A wheal diameter of 3 mm or more was considered positive [5].

ELISA/ELISA Inhibition Studies

Parietaria pollen was submitted to 5% aqueous extraction in PBS overnight at 4°C under stirring. The suspension was centrifuged at 20,000 g for 30 min at 4°C and the supernatant was filtered through a 0.22-mm filter. Protein content, measured according to Bradford [6] (BioRad, Milan, Italy), was 1,570 µg/ml.

IgE reactivity to both *Parietaria* pollen and natural profilin purified from *Phleum* pollen by affinity chromatography [7, 8] was measured by ELISA. ELISA was carried out using 0.5 µg/well of protein diluted in coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6) and coated onto the wells of 96-microwell ELISA plates (Maxisorp Nunc, Roskilde, Denmark) overnight at 4°C. 100 µl of serum were added to the wells and incubated for 2 h at 25°C. After washings, bound specific IgE were detected by adding a peroxidase-conjugated anti-human-IgE goat serum (1:1,000, HRP conjugated; Euroclon, Celbio, Milan, Italy) and developing colorimetric reaction using TMB/H₂O₂ as substrate. Results were expressed as optical density units (OD). Based upon the mean value $\pm$ 2 SD of 10 normal sera, OD values >570 were considered positive. Sera from 5 birch-pollen-allergic patients monosensitized to the major birch pollen allergen (i.e. showing IgE to rBet v 1 but not to rBet v 2 by CAP system), a pool of sera from patients monosensitized to *Parietaria*, and a pool of normal sera were tested against *Phleum* profilin as a control; the two serum pools and the serum from 1 patient monosensitized to Bet v 1 were tested against whole *Parietaria* extract as well.

ELISA inhibition studies were carried out on sera from 12 patients showing IgE reactivity to *Parietaria* pollen. In these experiments IgE reactivity to *Phleum* profilin was measured before and after absorption of 65 µl of serum with 65 µl of whole *Parietaria* pollen extract (1:1, 1:3, and 1:9 for 2 h at 25°C). IgE reactivity to both whole *Parietaria* pollen extract and *Phleum* profilin was measured also after absorption of sera with 5 µg of the Par j 1/Par j 2 fraction of pellitory pollen purified by affinity chromatography using a specific monoclonal antibody. Based upon the findings with control inhibitors (house dust mite extract), reductions >75 , 50–75, 25–50, and $<25\%$ of IgE reactivity to profilin were considered as total, moderate, slight, and no inhibition, respectively. In order to exclude the possibility that low inhibition values could be the consequence of an imbalance between high concentrations of profilin-specific IgE and a low profilin concentration in *Parietaria* extract, most experiments where little or no inhibition of IgE reactivity to *Phleum* profilin by *Parietaria* extract was found were repeated diluting patients' sera 1:2.

Results

Parietaria and Profilin Reactivity

IgE to rBet v 2, whole *Parietaria* pollen extract, and *Phleum* profilin as well as results of SPT to *Parietaria* pollen are shown in table 1. Sera from all 36 patients showed strong IgE reactivity to *Phleum* profilin. Only 14/36 (39%) patients had a positive SPT to *Parietaria* pollen extract. ELISA with whole *Parietaria* pollen extract was positive in 17/36 (47%) patients: this proportion included all patients showing positive SPT to *Parietaria* extract and 3 further SPT-negative patients (table 1).

Inhibition Experiments

Table 2 shows the results of ELISA inhibition experiments. Inhibition of IgE reactivity to *Phleum* profilin by either whole *Parietaria* pollen extract or the Par j 1/Par j 2 fraction of *Parietaria* pollen was assessed in sera from 12 and 8 of the 17 *Parietaria* reactors, respectively (table 1). Absorption of sera with undiluted whole *Parietaria* pollen extract caused an inhibition of IgE reactivity to *Phleum* profilin in 8/12 (66%) cases (table 2). In 4/12 (33%) cases *Parietaria* pollen did not cause any inhibition of IgE reactivity to *Phleum* profilin. However, if sera showing little or no inhibition were diluted, significant inhibition of IgE reactivity to *Phleum* profilin was observed in 5/5 (100%) cases (table 2). Absorption of sera with the Par j 1/Par j 2 fraction of *Parietaria* pollen did not cause any reduction of IgE reactivity to *Phleum* profilin in 8/8 (100%) sera (table 2).

ELISA inhibition experiments performed using whole *Parietaria* pollen extract as substrate and the Par j 1/Par j 2 fraction as inhibitor were carried out on sera from 9

Table 1. IgE reactivity to birch and grass profilin, and to *Parietaria* pollen and SPT results

Patient No.	A	B	C	D
1	2.62	3,535	–	145
2	18.8	3,894	–	356
3	0.74	3,877	+	1,366
4	2.62	3,484	–	200
5	14.8	3,900	+	3,776
6	1.17	3,141	+	1,541
7	9.52	3,736	–	224
8	12.6	3,866	–	170
9	6.11	3,910	–	325
10	1.95	2,966	–	178
11	3.74	3,824	–	168
12	0.58	2,091	+	1,825
13	1.10	2,782	–	208
14	17.9	3,862	+	670
15	2.73	3,841	–	1,141
16	4.36	3,634	–	484
17	1.12	3,750	+	1,274
18	21.4	3,912	+	3,558
19	9.80	3,783	+	587
20	8.47	3,926	–	314
21	4.84	3,777	–	1,272
22	0.46	3,034	–	194
23	4.97	3,775	–	408
24	6.46	3,835	–	164
25	6.33	3,784	+	1,582
26	2.14	3,728	–	188
27	9.85	3,773	+	3,862
28	2.30	3,822	+	1,300
29	62.8	4,000	+	2,706
30	2.07	3,823	–	361
31	4.39	4,000	+	1,944
32	1.04	2,465	–	180
33	63.9	3,887	–	3,567
34	0.98	2,923	+	3,613
35	6.81	3,674	–	327
36	0.72	1,458	–	164
Bet1		526		
Bet2		259		
Bet3		425		
Bet4	0	309	–	208
Bet5		215		
Neg		202	–	324
Par		527		3,609

A = IgE reactivity to rBet v 2 by CAP system (kU/l); B = IgE reactivity to *Phleum* profilin (OD; positive if >570); C = SPT with *Parietaria*; D = IgE reactivity to whole *Parietaria* extract (OD; positive if >570); Bet1–5 = control sera from patients monosensitized to birch pollen; Par = serum pool from patients monosensitized to *Parietaria*; Neg = pool of normal sera.

Table 2. ELISA inhibition experiments

Patient No.	A	B	C	D	E
3	3,877	46% (60%)	0%	1,366	8%
5	3,900	39% (83%)	0%	3,776	77%
6	3,141	57%	0%	1,541	76%
12	2,091	70%	0%	1,825	81%
15	3,841	79%	0%	1,411	ND
17	3,750	ND	ND	1,274	84%
18	3,912	7% (ND)	0%	3,558	ND
21	3,777	73%	0%	1,272	ND
27	3,773	23% (79%)	ND	3,862	80%
28	3,822	82%	8%	1,300	54%
29	4,000	0% (68%)	ND	2,706	55%
33	3,887	4% (84%)	ND	3,567	ND
34	2,923	64%	ND	3,613	53%

A = IgE reactivity to *Phleum* profilin (OD); B = percent inhibition of IgE reactivity to *Phleum* profilin by whole *Parietaria* pollen extract (the inhibition observed in experiments performed diluting sera 1:2 is shown in parentheses); C = percent inhibition of IgE reactivity to *Phleum* profilin by Par j 1/Par j 2 fraction; D = IgE reactivity to whole *Parietaria* extract (OD); E = percent inhibition of IgE reactivity to whole *Parietaria* extract by Par j 1/Par j 2 fraction; ND = not done.

out of 17 *Parietaria* reactors. Absorption of sera with Par j 1/Par j 2 fraction inhibited IgE reactivity to whole *Parietaria* pollen extract in 8/9 (89%) cases; inhibition was total in 5 cases and moderate in 3 cases. In 1 case (patient 3) very low inhibition was observed.

Discussion

Several studies have shown that profilins from botanically unrelated plants share IgE-binding epitopes. The immunological cross-reactivity between profilins in birch, grass, and mugwort pollen was first observed by Valenta et al. [2], and subsequently confirmed by other groups [9–11]. This study further confirmed these observations, since all patients showing IgE reactivity to birch profilin were strongly reactive against *Phleum* profilin as well. Surprisingly enough, none of the earlier studies of profilin cross-reactivity included *Parietaria* pollen; a possible reason for this is that *Parietaria* represents a relevant airborne allergen in the Mediterranean basin but not in Northern European countries where all those studies were carried out. By extrapolation, subsequently *Parietaria* profilin has been considered as cross-reacting with profil-

ins from phylogenetically distant botanical families. A Spanish study showed that amino acid sequences deduced from cDNA clones from *Parietaria* profilin share high identity with other plant profilins, including *Olea europaea*, *Phleum pratense*, and *Helianthus annuus* [4]. However, a high identity in amino acid sequence does not necessarily mean immunological cross-reactivity as well. In fact, by the use of a monoclonal antibody to sunflower profilin the same Spanish group observed some differences in cross-reactivity patterns between *O. europaea*, *P. pratense*, *Cynodon dactylon*, *Parietaria judaica*, *Mercurialis annua*, and *Betula verrucosa* [12]. More recently, Mari [13] found that only 50% of patients with multiple pollen sensitizations (defined as skin reactivity to at least 12 out of 24 botanically unrelated pollen species) had a positive SPT to *Parietaria* suggesting the lack of cross-reacting epitopes in this pollen. Finally, T-cell lines specific for *P. judaica* profilin showed no cross-reactivity to homologous T-cell lines specific for *Phleum* profilin [14]. All these findings were in keeping with our preliminary observation that SPT to *Parietaria* pollen is often negative in patients showing multiple pollen sensitization, and suggested that *Parietaria* profilin might not always cross-react with profilins from other plant species. In the

present study sera from less than 50% of patients with defined hypersensitivity to birch and grass profilin showed immunological cross-reactivity to *Parietaria*, thus confirming this hypothesis. A very recent study by Stumvoll et al. [15] showed that in patients living in the non-Mediterranean areas sensitized to mugwort and ragweed, IgE reactivity to *Parietaria* pollen is frequently caused by cross-reactive allergens, including profilin and calcium-binding protein. However, the findings of that study and of the present one are not mutually exclusive, as Stumvoll et al. [15] selected their patients in different geographic areas on the basis of IgE reactivity to *Parietaria*, whereas our patients were selected on the basis of IgE reactivity to birch profilin, Bet v 2. Their study was not designed to detect the prevalence of *Parietaria* sensitization among patients sensitized to profilin.

In conclusion, this study shows that: (i) only less than 50% of patients hypersensitive to birch and grass profilin recognize this cross-reacting, ubiquitous allergenic protein in *Parietaria* pollen, and (ii) most of those who react to *Parietaria* profilin are sensitized also to the major, specific pellitory allergens. This information may have practical relevance when the prescription of specific immunotherapy is considered.

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