

Profilin as an Aeroallergen by Means of Conjunctival Allergen Challenge with Purified Date Palm Profilin

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Key Words

Aeroallergen, conjunctival • Challenge • Profilin

Abstract

Background: The role of profilin as a food allergen is well established, but little research has been done about its ability to elicit respiratory disease. Profilin is considered more of a confounding allergen on skin testing with whole pollen extracts than other airborne allergens. Our aim was to find out whether or not profilin can cause symptoms in sensitized individuals, which would be compatible with its role as an airborne allergen. **Methods:** We performed conjunctival allergen challenges with date palm profilin in a series of consecutive pollen-allergic patients with rhino-conjunctivitis, divided in two groups: profilin sensitized ($n = 17$) and not sensitized ($n = 14$), who served as controls. We investigated the possible association between profilin sensitization and profilin allergy in these groups of patients. **Results:** None of the patients from the not profilin-sensitized group had a positive result in conjunctival allergen challenges. In contrast, 65% of profilin sensitized patients had a positive conjunctival allergen challenge and were considered allergic to profilin. We found a significant statistical association between being profilin allergic and being profilin sensitized

($\chi^2 = 10.39$, $p < 0.005$). **Conclusions:** Profilin seems to work as an aeroallergen in a significant proportion of profilin-sensitized patients. This might explain the uselessness of conjunctival challenges with whole pollen extracts to disclose genuine sensitization. In the future, the possibility of quantifying this allergen in pollen immunotherapy vaccines should be considered.

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Introduction

Profilin is a protein that exists in all eukaryotic cells, being highly conserved in vegetable species [1, 2]. Its ability to cause oral allergy syndrome is well known [3, 4]. Nevertheless, its role as an aeroallergen has been scarcely investigated [5].

Several studies have found that the prevalence of profilin sensitization among pollen-allergic patients ranges between 20 and 40% [6–9]. So, although it is not a major allergen, its importance should not be underestimated. Profilin-sensitized patients have a positive response by skin prick test and specific IgE determination when whole pollen extracts are used. This fact complicates the diagnosis and an adequate immunotherapy choice, al-

Table 1. Clinical features of the studied population

No.	Gen-der	Age	RC	RC and A	OAS	Evo-lution years	Lolium spt	Plan-tago spt	Betula spt	Profilin spt
1	F	11	+	-	-	5	+	+	-	+
2	M	13	+	-	-	7	+	+	-	+
3	M	27	+	-	-	14	+	+	+	+
4	F	38	+	-	-	20	+	+	+	+
5	F	24	+	-	-	12	+	+	-	+
6	M	13	+	+	-	7	+	+	-	+
7	F	41	+	+	+	35	+	+	-	+
8	M	35	+	-	-	19	+	+	-	+
9	F	15	+	-	-	3	+	+	+	+
10	F	15	+	-	+	12	+	+	+	+
11	F	19	+	-	+	2	+	+	+	+
12	M	7	+	-	-	5	+	-	-	+
13	M	11	+	-	-	2	+	+	+	+
14	F	30	+	-	-	25	+	+	+	+
15	F	46	+	+	-	8	+	+	-	+
16	F	16	+	-	-	3	+	+	-	+
17	M	31	+	-	-	6	+	+	-	+
18	M	21	+	-	-	10	+	-	-	-
19	F	8	+	-	-	4	+	-	-	-
20	M	5	+	+	-	3	+	+	-	-
21	M	14	+	-	-	1	+	-	+	-
22	F	9	+	+	-	2	+	-	-	-
23	M	16	+	+	-	3	+	+	+	-
24	F	18	+	-	-	10	+	+	-	-
25	F	12	+	-	-	2	+	+	-	-
26	F	13	+	+	-	1	+	-	-	-
27	M	13	+	-	-	3	+	+	+	-
28	F	21	+	-	-	2	+	-	-	-
29	F	43	+	-	+	3	-	-	+	-
30	F	40	+	+	+	30	+	+	+	-
31	F	35	+	+	-	2	+	+	-	-

RC = Rhino-conjunctivitis; A = asthma; OAS = oral allergy syndrome; spt = skin prick test.

though since component-resolved diagnosis has been put into practice, this problem has been solved to a major extent. In addition, it is possible that profilin could cause respiratory symptoms and thus act as a real aeroallergen. This would imply the need to control its presence and concentration in immunotherapy. Profilin is an allergen found in many pollen sources and theoretically it might behave nearly like a perennial allergen. For these reasons, the main objective of this work was to find out whether or not profilin can act as an airborne allergen.

Material and Methods

Patients

Between the first week of November 2009 and the first week of March 2010, all patients with a compatible history of pollen-allergic disease, rhino-conjunctivitis, or rhino-conjunctivitis and asthma, confirmed by a standard allergic evaluation that included the skin prick test with a battery of common aeroallergens (*Lolium perenne*, *Phleum pratense*, *Plantago lanceolata*, *Parietaria judaica*, *Betula verrucosa*, *Olea europaea*, and *Cupressus arizonica*), were offered entry into the study. Patients sensitized to perennial aeroallergens and those who had received any pollen extract immunotherapy in the last 5 years were excluded. None of the potential selected patients rejected inclusion into the study. The total number of patients recruited was 31. Of these patients, 17 were profilin sensitized (PS group), as determined by skin testing or specific IgE. The remaining 14 patients were not profilin sensitized (NPS group) and had negative results to skin tests and specific IgE determination. The patients, or parents in cases of minors, gave their written informed consent and the study was approved by the Hospital Ethical Committee. The main features of the patients are summarized in table 1.

Profilin Purification

Natural profilin from date palm pollen (nPho d 2) was purified from the pollen extract by affinity chromatography with poly-L-proline Sepharose [10]. The purity of the preparation was checked using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), mass spectrometry, and amino acid analysis. The concentration of protein was determined by Lowry's method [11].

In vivo Tests

Skin prick tests were performed according to EAACI methodology [12] with a standard battery of commercial extracts (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Glycyphagus domesticus*, *Chortoglyphus arcuatus*, *Lolium perenne*, *P. pratense*, *P. lanceolata*, *P. judaica*, *B. verrucosa*, *O. europaea*, *C. arizonica*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, cat epithelium, dog epithelium, cow epithelium, rabbit epithelium, horse epithelium, and latex) and purified natural date palm profilin (nPho d 2; 50 µg/ml; ALK-Abelló, Madrid, Spain). The tests were considered positive when there was a wheal ≥ 3 mm.

Conjunctival allergen challenge (CAC) was performed in all of the 31 selected patients, during an asymptomatic period, with increasing concentrations of purified nPho d 2, the same preparation used for the prick test, except that phenol and glycerol were not present. We used nPho d 2 as a general source of profilin and supposed that none of the patients were sensitized to date palm pollen. CAC started at 0.5 µg nPho d 2 per ml, and the concentrations 5 and 50 µg/ml were used afterwards when there was a previous negative or insufficient response. The response was considered positive when it met the Abelson criteria [13] with minimum changes (minimal score of 2 for itching and redness) and by a double-blind placebo-controlled (saline) method. The top achievable score is 14. Table 2 shows the symptoms scoring system.

The positive cases were labeled as profilin allergic (PA) and the negative ones as not profilin allergic (NPA).

Table 2. CAC: scoring system*Itching (to be graded by the patient)*

- 0 None
- 1 Mild (intermittent tickling sensation)
- 2 Moderate (continual awareness but without the desire to rub)
- 3 Severe (continual awareness with the desire to rub the eyes)
- 4 Incapacitating itching (subject insists on rubbing eyes)

Redness

- 0 None
- 1 Mildly dilated blood vessels
- 2 Moderate dilation of blood vessels
- 3 Severe, numerous, and obvious dilated blood vessels
- 4 Extremely severe. Large, numerous dilated blood vessels

Chemosis

- 0 None
- 1 Mild (visually visible)
- 2 Moderate (visually very evident, raised conjunctiva)
- 3 Severe (ballooning of conjunctiva)

Tearing

- 0 None
- 1 Mild tearing (eyes feel slightly watery)
- 2 Moderate (blows nose occasionally)
- 3 Severe (tears rolling down cheeks)

Positive test: minimal score 2 on itching and redness. Maximal score: 14.

In vitro Test

Specific IgE to recombinant *P. pratense* pollen profilin (rPhl p 12) was determined using the ImmunoCAP system (Phadia, Uppsala, Sweden). In those patients who finally entered the study, specific IgE to natural date palm pollen profilin was also performed (nPho d 2, ADVIA-Centaur system; ALK-Abelló). In both determinations a cutoff value of 0.35 kU/l was considered.

It should be stressed that when we talk about specific IgE, we will refer to IgE to rPhl p 12.

Statistical Methods

The χ^2 test with Yates' correction was applied to the CAC results in order to compare proportions. Spearman's rank order correlation was performed between the IgE results to rPhl p 12 and those to nPho d 2.

Results

In the NPS group ($n = 14$), the CAC was negative in all of the patients, with a score of 0 for saline and profilin.

In the PS group, all patients ($n = 17$) had a positive prick test and 13/17 had a positive specific IgE. No patient with positive specific IgE and a negative prick test was found.

The CAC was positive in 11 cases (65%), achieving an average score of 8.73 ± 2.61 for profilin and 0 for saline.

From these 11 patients (PA group), in 3 cases the CAC was positive at 5 $\mu\text{g/ml}$ and in the other 8 the CAC was positive at 50 $\mu\text{g/ml}$. Of these last patients, 7 reacted at 5 $\mu\text{g/ml}$ but did not reach the minimum score required to be considered positive. All patients had a score of 0 at the concentration of 0.5 $\mu\text{g/ml}$.

Among the 6 PS cases with negative CAC tests, 4 reacted at 50 $\mu\text{g/ml}$ but they did not reach the demanded criteria, only achieving an average score of 3 for profilin and 0 for saline. Both of the 2 remaining cases, who obtained a score of 0 in the CAC, had a negative specific IgE. Among the 4 patients who reacted in an insufficient way, 2 had a negative specific IgE (fig. 1).

We found, using the χ^2 test with Yates' correction, a $p < 0.005$ statistical significance for the association between being PA and being PS.

A high Spearman correlation coefficient between IgE to rPhl p 12 and to nPho d 2 was obtained ($r = 0.873$, $p < 0.001$) (fig. 2).

Discussion

The role of profilin as a food allergen has been extensively studied, but little is known about the possibility of it acting as an aeroallergen. We chose the CAC test because the conjunctival mucous membrane is a target regularly involved in pollen-allergic diseases. In fact, the 31 challenged cases suffered from conjunctivitis. Although CAC does not reproduce the real pollen exposure scenery and we cannot assure that the PA patients would suffer symptoms from profilin with natural pollen exposure, we think CAC is a reliable technique to approach this issue.

In a study based on symptoms questionnaires, the authors found that pollen-allergic patients with profilin sensitization had higher symptom and medication scores during pollen season [14].

Asero et al. [8] split the year into 3 pollination periods (late February to mid-May, late April to mid-July, and mid-August to late-September) and found among 40 PS patients that 10% of them suffered from respiratory symptoms in the 3 periods, 35% in 2 periods, and 55% in only 1 period. The conclusion was that the clinical relevance of panallergen sensitization (profilin and/or polcalcic) is often limited. The same group [6] studied a population of 200 pollen-allergic patients and found that 30% were sensitized to date palm profilin. In this subgroup, the skin tests showed a negative result to whole pellitory

Fig. 1. Results of the CAC with nPho d 2 extract at 5- and 50- μ g/ml concentrations in the profilin-sensitized patients. The line shows the specific IgE obtained to rPhl p 12.

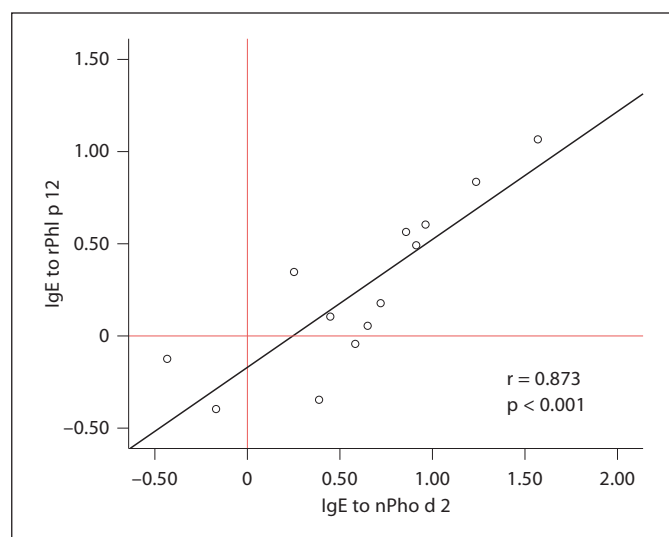
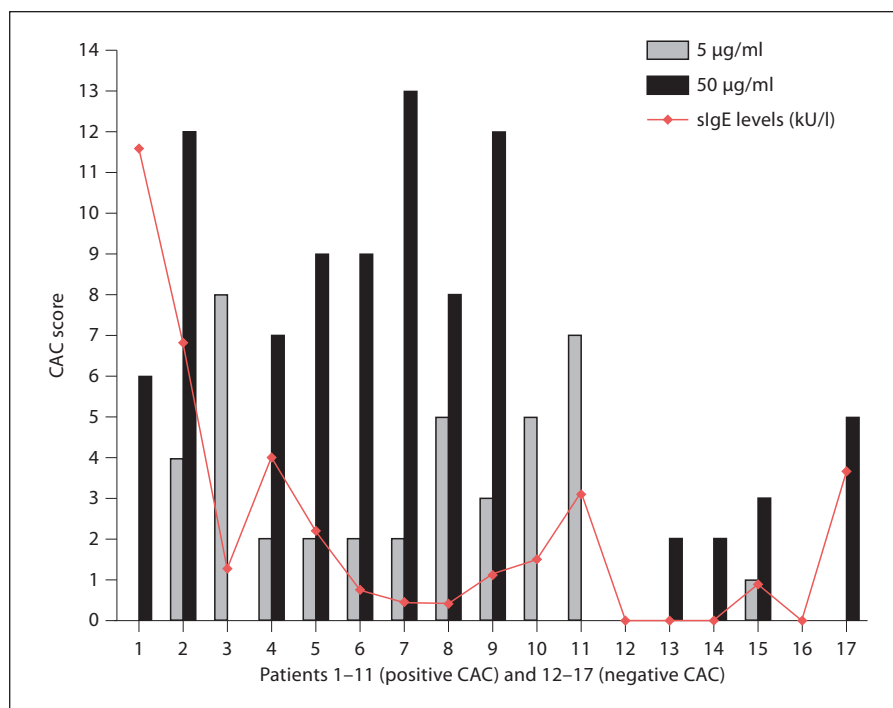


Fig. 2. Correlation between IgE to nPho d 2 and rPhl p 12. Log scale. Spearman's correlation coefficient, $r = 0.873$.

and cypress extract in 43 and 55% of the cases, respectively, suggesting partial cross-reactivity among these profilin sources. In a previous study [15], the same authors concluded that less than a half of grass and birch profilin-sensitized patients react to pellitory profilin.

Radauer et al. [16], studying profilin epitopes from 6 different sources (timothy, mugwort, celery, bell pepper, birch, and latex) and employing IgE inhibition assays, found that up to 60% of profilin epitopes are species specific and concluded that, although the high homology accounts for using only one profilin for diagnosis, the fine specificity of IgE pointed to diverse epitopes which might influence the clinical manifestations of sensitization. All of these data indicate that profilin-allergic patients do not necessarily present symptoms throughout the entire pollen season.

Our results show that, in 65% of PS patients, profilin is able to cause symptoms according to the CAC score. This percentage would rise to 88% (15/17) if any positive result in the CAC test were considered. We think these low CAC scores are significant since all of the patients in the NPS group had a profilin CAC score of 0 and in the PS group the saline CAC score was 0, too, ruling out un-specific reactions.

Our area, Lugo, is in the northwest of Spain. It has humid and cold weather and is virtually devoid of date palm trees. We believe that most of the patients acquired their profilin sensitization by grass pollen exposure since in our area grasses are clearly the most common cause of seasonal rhino-conjunctivitis. We used date palm profilin for skin testing and the CAC test due its availability

and to the high homology and immunoreactivity that it shares with the grass one [17]. In agreement with this, a high correlation was found between the specific IgE to rPhl p 12 and that to nPho d 2.

Although the profilin preparation we used was highly purified, it might have contained traces of other palm allergens. However, the possibility that these allergen traces could cause a positive response in the CAC is very remote since the probability of sensitization to date palm allergens among our patients is negligible. This is corroborated by the fact that all NPS patients had a negative response in the CAC test. Therefore, we think profilin is an allergen which should be taken into account since it affects up to about 20–40% of pollen-allergic patients,

some of them possibly in a perennial way. Before the advent of component-resolved diagnosis, choosing an adequate immunotherapy for multiple pollen-sensitized patients was nearly a random task. The general impression regarding the use of CAC with whole pollen extracts, in order to elucidate a genuine sensitizer, is that the results are misleading, too. This might be related to a reaction to profilin (and probably to other panallergens) present in the different pollen extracts.

It is known that the acquisition of panallergen sensitization happens late in the evolution of pollen allergic disease, so a preventive strategy might be the early onset of immunotherapy. For that purpose, the availability of profilin standardized extracts might be necessary.

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