ORIGINAL ARTICLE

Preliminary Results of a Skin Prick Test–Based Study of the Prevalence and Clinical Impact of Hypersensitivity to Pollen Panallergens (Polcalcin and Profilin)

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Abstract

Background: Calcium-binding proteins (polcalcins) and profilin are cross-reacting panallergens that sensitize a minority of pollen-allergic patients. Their clinical relevance remains controversial.
Objective: To assess the clinical relevance of hypersensitivity to polcalcin and profilin detected by skin prick test (SPT) in a large group of pollen-allergic patients.
Methods: Two hundred pollen-allergic adults (101 men, 99 women; mean age 34 years) underwent SPT with 9 pollens present in the geographical area of the study. Hypersensitivity to panallergens was detected by SPT with date palm polcalcin and profilin. Allergy to birch and/or cypress, grass and/or pellitory, and ragweed and/or mugwort were associated with 3 symptomatic periods, respectively, late February to mid-May, late April to mid-July, and mid-August to late September.
Results: Sixteen (8%) patients reacted to date palm polcalcin; 7/7 (100%) corecognized the grass polcalcin Phl p 7 in vitro. Clinically, only 4 (25%) had symptoms in all 3 seasonal periods. Forty (20%) patients reacted to profilin; only 32 (80%) reacted to cypress, and 22 (55%) to pellitory. Only 4 (10%) patients had symptoms during all 3 seasonal periods. Six patients (3%) were cosensitized to both polcalcin and profilin.
Conclusions: The clinical relevance of hypersensitivity to pollen panallergens is often limited; many allergic patients have symptoms only during the central period, suggesting primary grass sensitization. Profilin-allergic patients often do not corecognize pellitory and cypress pollen. In vivo component-resolved diagnosis of seasonal respiratory allergies is a promising approach that might lead to cost reduction and a faster definition of pollen-allergic cases.

Key words: Pollen allergy. Calcium-binding proteins. Polcalcin. Cross-reactivity. Skin prick test.

Resumen

Introducción: Las proteinas ligantes de calcio (polcalcinas) y la profilina son panalérgenos causantes de la sensibilización de una minoría de pacientes polínicos. Su relevancia clínica está todavía en debate.
Objetivo: Estudiar la prevalencia de sensibilización alérgica a polcalcina y profilina, detectada por prueba cutánea, en un grupo de pacientes polínicos.
Métodos: Se realizaron pruebas cutanéas a 200 pacientes polínicos (M/V 101/99, edad media 34 años) con extractos de 9 pólenes presentes en su área geográfica. La sensibilización a panalérgenos se detectó mediante pruebas cutáneas con polcalcina y profilina de palmera. Los tres períodos sintomáticos (fin febrero-med mayo, fin abril-med julio y med agosto-fin septiembre) se asociaron con alergia a abedul y/o ciprés, a gramíneas y/o parietaria y ambrosia y/o artemisia, respectivamente.
Resultados: 16 (8%) pacientes dieron positivos a la polcalcina de palmera en prueba cutánea. 7/7 (100%) reconocieron in-vitro al alergéno Phl p 7, la polcalcina presente en gramíneas. Clínicamente, sólo 4/40 (10%) desarrolló síntomas durante los 3 períodos polínicos. Seis pacientes (3%) presentaron sensibilización tanto a polcalcina, como a profilina.
Conclusiones: La prevalencia de sensibilización a panalérgenos de pólenos es limitada; muchos pacientes tienen síntomas sólo durante el período central (abril-julio), lo que sugiere una sensibilización primaria a gramíneas. Los pacientes positivos a profilina, no presentan reactividad ni frente a polen de parietaria, ni al polen de ciprés. El diagnóstico in-vivo por componentes en alergias respiratorias estacionales, es un abordaje prometedor que puede llevarnos a la reducción de costes, así como a una nueva definición en los casos de sensibilizaciones múltiples.

Palabras clave: Polinosis. Proteinas ligantes de calcio. Polcalcina. Reactividad cruzada. Prueba cutánea.

Introduction

Calcium-binding proteins (polcalcins) have recently been identified as cross-reacting pollen panallergens [1], and, together with profilin, are considered markers of multiple pollen sensitization [2]. Polcalcins have been detected in pollen from trees (Fagales, Cupressaceae, and Oleaceae), grasses, and weeds [3-8], and the grass calcium-binding protein Phl p 7 seems to be the most cross-reactive [9]. Polcalcins are generally considered minor allergens, and about 10% of pollen-allergic patients are sensitized to them.

In clinical practice, patients with pollen allergy are frequently sensitized to several taxonomically unrelated allergens [2,10]. In the case of candidates for allergen-specific immunotherapy, the clinician has to establish whether sensitization to several pollens is the result of cosensitization to different allergen proteins, corecognition of homologous allergens, or both [4]. To this end, detection of immunoglobulin (Ig) E reactivity to panallergens and to major specific pollen allergens is essential. This can only be achieved in vitro, by measuring IgE that is specific for a single polcalcin (eg, Phl p 7) and a single profilin (eg, Bet v 2) using assays that are not available in all clinical settings. The lack of an inexpensive and easy technique to detect sensitization to polcalcins on a routine basis in the doctor's office is probably one of the main reasons why there are no large studies on the clinical relevance of these aeroallergens. Consequently, little is known about the clinical impact of polcalcins. A recent study based on a skin prick test (SPT) with purified profilin showed how much the availability of a purified protein allergen for in vivo testing might help the clinician better understand the clinical relevance of single sensitizations at the first visit [11]. The present study assessed the clinical relevance of hypersensitivity to polcalcin and profilin detected for the first time by SPT in a large group of pollen-allergic patients.

Patients and Methods

Patients

Consecutive adult patients with a clinical history of pollen allergy attended recently at the allergy center of the Clinica San Carlo, Paderno Dugnano, Italy were screened for hypersensitivity to polcalcin and profilin. We aimed to include 200 patients with clinically defined pollinosis. A history of rhinoconjunctivitis with or without asthma during the pollen seasons of this geographic area [12,13]—late February to mid-May, late April to mid-July, and mid-August to late September—was considered to be possibly associated with respiratory allergy to birch and/or cypress, grass and/or pellitory, and ragweed and/or mugwort, respectively. All patients were interviewed to ascertain the presence/absence of respiratory symptoms during the 3 different periods. When this was not clear, patients were asked to record and grade respiratory symptom severity using a visual analog scale and were re-evaluated after the end of the season (mid-October). About 20% of enrolled patients were also sensitized to perennial airborne allergens, including mites, *Alternaria*, and cat dander.

Skin Tests

Patients underwent SPT with commercial extracts of the pollens present in the geographical area of the study including grass, mugwort, ragweed, pellitory, plantain, birch olive (50 000 SBU/mL, all manufactured by Allergopharma, Reinbeck, Germany), *Platanus*, and cypress (30 HEP; ALK Abelló, Madrid, Spain). Hypersensitivity to the panallergens polcalcin and profilin was detected by SPT with purified date palm polcalcin and profilin (ALK Abelló). All SPT were performed using disposable 1-mm–tip lancets (ALK Abelló). Readings were taken at 15 minutes, and a mean wheal diameter of 3 mm or more was considered positive [14].

Purified Date Palm Profilin and Polcalcin SPT

Following preliminary experiments (carried out at ALK-Abelló Laboratories, Madrid, Spain) showing that IgE responses to date palm pollen could be ascribed either to profilin or polcalcin, and that no reactivity to lipid transfer protein (LTP), cross-reactive carbohydrate determinants, or glucanase was detected, natural profilin (Pho d 2) was purified from date palm extract by affinity chromatography with poly-L-proline Sepharose [15]; purity was checked using sodium dodecyl sulfate polyacrylamide–gel electrophoresis (SDS-PAGE), mass spectrometry, and amino acid analysis. The concentration of Pho d 2 in the extract was 50 µg/mL. Date palm polcalcin SPT was obtained using the same extract (total protein concentration of 500 µg/mL following the method of Lowry [16]) after subtraction of profilin. Protein identity was assessed by SDS-PAGE.

Assessment of Sensitivity of Polcalcin SPT

The sensitivity of polcalcin SPT was assessed in 7 patients whose sera had previously been shown to contain elevated levels (range, 4.3-23.5 kU_A/L) of IgE specific for the grass polcalcin Phl p 7 using ImmunoCAP (Phadia, Uppsala, Sweden).

Results

A total of 216 patients whose history suggested pollinosis were screened in order to obtain 200 patients with clinically defined pollen allergy. The study group had a mean age of 34 years (range, 5-72 years). The gender distribution was 101 men and 99 women.

The results are summarized in the Table. Polcalcin hypersensitivity was detected in 16/200 (8%) patients (9 men, 7 women; mean age, 37 years). Twelve (75%) patients who were allergic to polcalcin showed positive SPT results to all 9 pollen extracts tested; 2 reacted to 8 pollen extracts and 2 to only 6 pollen extracts. All 16 polcalcin-hypersensitive patients had a positive SPT result for grass, ragweed, birch, and olive extracts; 15 patients had a positive SPT result with mugwort and plantain pollen extracts; and 14 patients had a positive result for pellitory, cypress, and *Platanus*. Only 4/16 (25%) polcalcin-allergic patients had clinical symptoms in all 3 seasonal periods. Four patients reported respiratory symptoms during the first 2

Table. Hypersensitivity to Seasonal Airborne Allergens in the Study Population

Number of Allergen Souces Recognized	Number of Patients	Number of Patiens Sensitized to Polcalcin	Number of Patients Sensitized to Profilin
1	51	0	0
2	32	0	0
3	27	0	0
4	16	0	1
5	11	0	2
6	7	2	2
7	7	0	4
8	17	2	9
9	31	12	22
Total	200	16	40

periods, 2 during the second and the third period only, and the remaining 6 only during the central period. Regarding sensitivity of the in vivo test, all 7 (100%) patients whose sera contained IgE to Phl p 7 by Immuno-CAP had a positive SPT result with date palm polcalcin.

Hypersensitivity to the other pollen panallergen, profilin, was detected in a larger number of cases (40/200 [20%]; men 18, women 22; mean age, 32 years). Although most profilin-allergic patients had a positive SPT result with ≥8 pollen allergen sources, 9 patients reacted to a lower number of pollen extracts (1 patient to as few as 4) (Table). All 40 profilin-hypersensitive patients had a positive SPT result with grass pollen extract, 39 reacted to mugwort, ragweed, plantain, and olive, 38 to birch pollen, and 36 to plantain. Only 32 (80%) and 22 (55%) patients reacted to cypress and pellitory on SPT, respectively. Only 4/40 (10%) patients had respiratory symptoms during all 3 periods; 14 patients had symptoms during 2/3 periods (most of them during the first two periods); 22 patients had symptoms during only 1 period (the central period in 19 cases).

A total of 63 (31.5%) study patients were sensitized to ≥6 respiratory allergens. Interestingly, 12 were not allergic to panallergens; 4 reported respiratory symptoms during all 3 pollen periods, 5 had symptoms only during the middle (grass pollen) period, 2 during the last 2 periods, and 1 during the first and the last period.

Six patients (3%) were cosensitized to both polcalcin and profilin; 5/6 reacted to 9/9 pollen extracts on SPT, whereas 1 reacted only to 6 extracts.

Discussion

The present study investigated the prevalence and clinical relevance of hypersensitivity to pollen panallergens, namely polcalcin, a calcium-binding protein, and profilin. The main novelty of this work is that polcalcin IgE reactivity was detected in vivo by SPT using a specifically prepared date palm extract. The specific concentration of polcalcin in the natural palm pollen extract used to carry out the SPT was not known, as the polcalcin reagent was obtained after removing profilin from a whole pollen extract (protein 0.5 mg/mL) containing only polcalcin and profilin as allergens. While the specificity of the polcalcin SPT was not assessed due to the high cost—this would have meant measuring Phl p 7–specific IgE by Immuno CAP in all study patients before SPT—its sensitivity was rather good, as 7/7 (100%) patients with high levels of IgE to Phl p 7 had a positive SPT result with polcalcin. The prevalence of sensitization to the 2 panallergens found in the study group did not differ significantly from that reported in the literature [2,17]. Altogether, a quarter (50/200) of the study patients were sensitized to at least 1 pollen panallergen. This fact clearly underlines the potential value of component-resolved diagnosis in areas where many allergen sources are present.

The clinical relevance of the 2 panallergens remains controversial; the findings of the present study seem to suggest that it is often limited. In fact, if panallergens were clinically relevant, hypersensitive patients would be expected to suffer from allergic symptoms throughout the pollen season (in this area from February to late September). However, this was the case in only a few patients who were hypersensitive to both polcalcin and profilin. A large proportion of panallergen-sensitized patients reported significant respiratory symptoms in only 1 of the 3 pollen periods (most frequently the central period), suggesting primary grass pollen sensitization. Interestingly, 12 patients sensitized to multiple allergens did not show any skin reactivity to either polcalcin or profilin. We cannot rule out the possibility that these patients were sensitized to panallergens but were not correctly diagnosed due to the lack of sensitivity of the SPT extracts used. However, in view of the reported prevalence of allergy to polcalcin and profilin among pollen-allergic patients, this is rather unlikely, and the fact that these patients were allergic to several different allergens seems a more likely explanation.

This study confirmed previous observations that profilin-allergic patients often do not corecognize pellitory and, to a lesser extent, cypress [11], possibly due to epitopic differences in profilins from these allergen sources.

An interesting aspect that has received little attention is the existence of patients who are cosensitized to both profilin and polcalcin (3% in our study). The sensitization of these patients should be clarified by component-resolved diagnosis so that allergen-specific immunotherapy can be prescribed.

Although presently limited to only 2 pollen panallergens, in vivo component-resolved diagnosis of seasonal respiratory allergy is already a very promising approach that will hopefully make allergy diagnosis less expensive than in vitro tests and provide a faster diagnosis in patients who are hypersensitive to several allergens.

In conclusion, this preliminary trial carried out at a doctor's office provided several interesting findings. SPT results and specific IgE measurements have recently been compared in a large multicenter trial systematically investigating the performance of SPT with polcalcin [18].

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