

Do Skin Prick Test and In Vitro Techniques Diagnose Sensitization to Peach Lipid Transfer Protein and Profilin Equally Well in Allergy to Plant Food and Pollen?

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■ Abstract

Objective: To compare the skin prick test (SPT) with in vitro techniques (single and multiplex fluorescence enzyme-immunoassay [FEIA]) for detecting sensitization to profilin and lipid transfer protein (LTP).

Methods: We retrospectively studied 181 patients with pollen and/or plant food allergy and 61 controls. SPT was performed with date palm profilin (Pho d 2) and peach LTP (Pru p 3), and specific IgE (sIgE) to Phl p 12 and Pru p 3 was analyzed using single FEIA and microarray.

Results: Fifteen of 201 patients with negative results for LTP in the SPT were sensitized to this allergen in the in vitro tests, and 18 of 41 patients with positive results for LTP in the SPT were not sensitized according to the in vitro tests. Seventeen of 186 patients with negative results for profilin in the SPT were sensitized to Phl p 12 by serum sIgE, and 30 out of 56 patients with positive results for profilin in SPT were not sensitized to Phl p 12 according to the other tests. Moderate agreement was observed between the 3 techniques studied.

Conclusions: SPT is a sensitive technique for detecting sensitization to LTP and profilin. Its results are similar to those of in vitro techniques, especially in patients with negative SPT results for peach LTP and palm tree profilin.

Key words: Lipid transfer protein. Profilin. Skin prick test. sIgE. Diagnosis.

■ Resumen

Objetivo: Comparar las pruebas cutáneas prick (PC) con técnicas in vitro (fluoro enzimoimmunoensayo –FEIA- en detección única y múltiple) para detectar sensibilización a profilina y a LTP.

Métodos: Se estudiaron retrospectivamente 181 pacientes con alergia a polen y a alimentos vegetales y 61 controles. Se realizaron PC frente a profilina de palmera (Pho d 2) y LTP de melocotón (Pru p 3) y se analizó la IgE específica a Phl p 12 y Pru p 3 por FEIA y por micromatriz de proteínas alergénicas.

Resultados: Quince de los 201 sujetos con PC negativa a LTP mostraron sensibilización a este alérgeno mediante IgE específica sérica y en 18 de 41 con PC positivas a LTP no se observó esta sensibilización por otras técnicas. Diecisiete de los 186 sujetos con PC negativa a profilina detectaron IgE específica sérica frente a Phl p 12 y en 30 de los 56 con PC positiva a profilina no se objetivó sensibilización a Phl p 12 en suero. Se observó un acuerdo moderado entre las tres técnicas estudiadas.

Conclusiones: La PC frente a LTP y profilina es un método sensible detectando estas sensibilizaciones y muestra un acuerdo aceptable con las técnicas in vitro, especialmente en los pacientes con negatividad de la PC frente a LTP y a profilina.

Palabras clave: Proteínas de transferencia de lípidos. Profilina. Pruebas cutáneas. IgE específica. Diagnóstico.

Introduction

Panallergen sensitization remains a diagnostic challenge, especially in pollen allergy and plant food allergy. Profilin and lipid transfer protein (LTP) are the most prevalent panallergens involved in allergy to pollen and plant food in the Mediterranean area [1-4]. In recent years, skin prick test (SPT) and determination of serum specific IgE (sIgE) have become the most commonly used techniques in the allergy workup. Component-based diagnosis is widely applied for determination of sIgE, not only in a single determination such as fluorescence enzyme immunoassay (FEIA, eg, ImmunoCAP), but also in multiplex platforms such as the allergen microarray Immuno Solid-phase Allergen Chip (ISAC). In addition, purified and enriched components (eg, palm tree profilin and peach LTP) are also available for SPT. Although the availability of such a wide range of diagnostic techniques is useful, it can also lead to confusion owing to the amount of data generated. Moreover, clinicians need to have a detailed knowledge of how each test performs when diagnosing sensitization in order to optimize resources. Thus, the aim of this study was to compare an in vivo technique (SPT) with 2 in vitro techniques (ImmunoCAP and ISAC CRD103) for assessment of sensitization to profilin and peach LTP in patients allergic to pollen and plant food.

Methods

Patients

We retrospectively studied consecutive outpatients with pollen allergy and/or plant food allergy attending the Clinica Universidad de Navarra in Pamplona, Spain. A complete clinical history was taken to document respiratory symptoms, and a questionnaire administered to determine tolerance to the most frequently consumed plant foods in our area. Controls were included consecutively when plant food allergy and pollen allergy were excluded. SPT was performed with peach LTP and palm tree profilin, and sIgE was determined using ISAC CRD103 microarray.

Skin Prick Tests

All patients underwent SPT to peach (30 µg/mL of natural [n] Pru p3) and purified palm profilin (50 µg/mL of nPho d 2) (ALK-Abelló). SPT was also performed with a battery of aeroallergens containing the most prevalent pollens in Spain (*Phleum pratense*, *Cynodon dactylon*, *Olea europaea*, *Cupressus arizonica*, *Betula verrucosa*, *Plantago lanceolata*, *Platanus acerifolia*, *Salsola kali*, and *Parietaria judaica*) (ALK-Abelló), as well as a panel of food allergens (wheat, nuts, fruits, legumes, egg, milk, fish, and shellfish) (Bial-Aristegui). SPT involved puncture with a standard 1-mm-tip lancet (ALK-Abelló) on the volar surface of the forearm. Sodium chloride (0.9%) and histamine hydrochloride (10 mg/mL, ALK-Abelló) served as negative and positive controls, respectively. SPTs were read after 15 minutes. Wheals of ≥ 3 mm in diameter were considered positive, as recommended by the guidelines of the European Academy of Allergology and Clinical Immunology [5]. SPTs were always performed by the same experienced nurses.

Specific IgE Against Panallergens

sIgE was quantified against recombinant peach LTP (rPru p 3) using FEIA (ImmunoCAP 250, Thermo Fisher Scientific). Given that the date palm tree profilin Pho d 2 is not available for this platform, sIgE against grass pollen profilin rPhl p 12 was also measured using ImmunoCAP. Values ≥ 0.35 kU_A/L were considered positive.

sIgE was also determined against rPhl p 12 and rPru p 3 using the ISAC allergen microarray immunoassay ISAC ImmunoCAP version CRD103 (Thermo Fisher Scientific), which also includes other LTPs (nArt v 3, rCor a 8, and rPar j 2) and profilins (rBet v 2, nOle e 2, rHev b 8, and rMer a 1). Values ≥ 0.3 ISU were considered positive.

Statistical Analysis

Quantitative variables were described as mean (SD) or median (IQR), depending on whether the data were distributed normally or not (Kolmogorov-Smirnov test); qualitative variables were described as frequency (percentage). Differences between groups for quantitative variables were evaluated using analysis of variance (ANOVA) or the Kruskal-Wallis test. The chi-square test (or Fisher exact test when needed) was used to compare proportions.

Diagnostic agreement between the techniques was expressed in qualitative terms (positive/negative) by calculating the kappa index (κ) and interpreting the results based on the Fleiss criteria [6], as follows: $\kappa < 0.4$, poor; $0.4 \leq \kappa \leq 0.75$, moderate; and $\kappa > 0.75$, excellent.

Agreement for microarray results (expressed in quantitative terms [ISU]) was also assessed in the determination of sIgE against different profilins and LTPs using microarray by calculating the intraclass correlation coefficient (ICC). The level of agreement using the ICC was expressed using the classification of Fleiss [7], as follows: very good, $ICC > 0.90$; good, $ICC = 0.71-0.90$; moderate, $ICC = 0.51-0.70$; mediocre, $ICC = 0.31-0.50$; and poor, $ICC < 0.30$.

All statistical calculations were performed using SPSS version 15.0 (SPSS Inc). *P* values of $< .05$ were considered significant.

Results

Characteristics of the Sample

The study sample comprised 242 participants (181 patients and 61 controls). Pollen allergy without plant food allergy was recorded in 107 patients, plant food allergy and pollen allergy in 47, and plant food allergy without pollen allergy in 27. Among the 74 patients with plant food allergy (47 with pollen allergy and 27 without pollen allergy), SPT revealed that 48 of the 61 controls had allergy to dust mite, dander, or latex and 13 were not sensitized to any of the study allergens. The demographic characteristics of the participants are shown in Table 1.

Sensitization to Peach LTP

SPT revealed sensitization to peach LTP in 13 of the 107 pollen-allergic patients without plant-food allergy (12%),

Table 1. Characteristics of the Sample

	Pollen Allergy	Pollen and Plant Food Allergy	Plant Food Allergy	Controls	
Number of patients	107	47	27	61	
Median (IQR) age, y	32.2 (20-40)	29 (19-39)	33.2 (16-46)	31.5 (18-42)	$P=.716$
Sex, % males	67.3	34	51.9	50	$P=.001^a$

^aChi-square. Males were more frequent than expected in pollen-allergic patients without food allergy and females more frequent than expected in patients with pollen and plant food allergy.

Table 2. Comparison of Patients Sensitized to Profilin and Peach LTP by the 3 Techniques

	ImmunoCAP Pru p 3				SPT Phl p 12	ImmunoCAP Phl p 12			
	Negative		Positive			Negative		Positive	
	ISAC Pru p 3 Negative	ISAC Pru p 3 Positive	ISAC Pru p 3 Negative	ISAC Pru p 3 Positive		ISAC Phl p 12 Negative	ISAC Phl p 12 Positive	ISAC Phl p 12 Negative	ISAC Phl p 12 Positive
SPT Pru p 3					SPT Phl p 12				
Negative	186	1	7	7	Negative	169	4	6	7
Positive	9	2	7	23	Positive	20	6	4	26

Abbreviations: LTP, lipid transfer protein; SPT, skin prick test.

12 of the 47 pollen and plant food-allergic patients (25%) and 12 of the 27 plant food-allergic patients without pollen allergy (44%). SPT also revealed that 4 controls were sensitized to peach LTP.

Among the 41 patients with a positive SPT to peach LTP, 23 had positive sIgE to Pru p 3 both by ImmunoCAP and by microarray. Seven showed serum sIgE against Pru p 3 only by ImmunoCAP and 2 only by microarray. Nine of the patients with positive results to peach LTP in the SPT had negative results against Pru p 3 in vitro. Seven of the 201 patients with negative results for peach LTP in the SPT had positive sIgE against Pru p 3 by both ImmunoCAP and microarray. Seven patients with negative results for peach LTP in the SPT had positive sIgE against Pru p 3 only by ImmunoCAP and 1 only by microarray.

The results for peach LTP with each of the 3 techniques are shown in Table 2.

Sensitization to Profilin

SPT revealed sensitization to palm tree profilin in 30 of the 107 pollen-allergic patients without plant-food allergy (28%), 18 of the 47 pollen and plant food-allergic patients (38%), and 4 of the 27 plant food-allergic patients without pollen allergy (15%). Four controls were sensitized to profilin by SPT.

Of the 56 patients with a positive SPT result to palm tree profilin, 26 had positive sIgE to timothy grass profilin by both ImmunoCAP and microarray. Four had serum sIgE against Phl p 12 only by ImmunoCAP and 6 only by microarray. Twenty of the patients with a positive SPT result for profilin showed negative results against Phl p 12 in vitro. Seven of the 186 patients with a negative SPT result for profilin had positive sIgE against Phl p 12 by both ImmunoCAP and microarray.

Six patients with negative SPT results for palm tree profilin had positive sIgE against Phl p 12 only by ImmunoCAP and 4 only by microarray.

The results for each of the 3 techniques with profilin are shown in Table 2.

Diagnostic Agreement Between Skin Prick Test and sIgE Assessed Using ImmunoCAP and ISAC With LTP

Moderate agreement was observed between SPT with peach LTP and sIgE to Pru p 3 determined by ImmunoCAP, as well as between SPT with peach LTP and sIgE against Pru p 3 by microarray. Indeed, moderate agreement was also observed for sensitization to Pru p 3 diagnosed by ImmunoCAP and microarray. These data are summarized in Table 3.

Poor agreement (ICC=0.328) was observed between the 4 LTPs present in the microarray (Pru p 3, Par j 2, Cor a 8, and Art v 3). This agreement improved significantly when LTP from *Parietaria* was excluded from the analysis (ICC=0.832) based on reported data [8].

Diagnostic Agreement Between Skin Prick Test and sIgE Assessed Using ImmunoCAP and ISAC With Profilin

Agreement between SPT with profilin and sIgE against Phl p 12 by ImmunoCAP and by microarray was analyzed. Moderate agreement was observed between SPT with profilin and ImmunoCAP with Phl p 12 and between SPT with profilin and microarray with Phl p 12. Moderate agreement was also observed for sensitization to Phl p 12 diagnosed by ImmunoCAP and microarray (Table 4).

Agreement between sIgE against the 5 profilins represented in the microarray was almost perfect (ICC=0.976).

Table 3. Agreement Between Tests in the Diagnosis of Sensitization to Lipid Transfer Protein

	SPT Pru p 3	ImmunoCAP Pru p 3
SPT Pru p 3		
NA		93.73%
PA		70.59%
κ		0.643
ISAC Pru p 3		
NA	94.15%	95.82%
PA	67.57%	77.92%
κ	0.618	0.738
ISAC Art v 3		
NA	91.39%	91.08%
PA	45.45%	46.38%
κ	0.374	0.382
ISAC Cor a 8		
NA	91.88%	92.52%
PA	33.96%	42.86%
κ	0.285	0.380
ISAC Par j 2		
NA	90.62%	89.86%
PA	12.77%	12%
κ	0.088	0.080

Abbreviations: NA, negative agreement; PA, positive agreement; SPT, skin prick test.

Table 4. Agreement Between Tests in the Diagnosis of Sensitization to Profilin

	SPT Pho d 2	ImmunoCAP Phl p 12
SPT Pho d 2		
NA		89.87%
PA		60.61%
κ		0.507
ISAC Phl p 12		
NA	90.91%	94.97%
PA	64.65%	76.74%
κ	0.558	0.717
ISAC Ole e 2		
NA	91.82%	96.94%
PA	70.48%	86.96%
κ	0.623	0.839
ISAC Hev b 8		
NA	92.80%	92.80%
PA	75.23%	75.23%
κ	0.680	0.741
ISAC Bet v 2		
NA	92.47%	94.55%
PA	75.00%	78.79%
κ	0.675	0.735
ISAC Mer a 1		
NA	93.80%	94.79%
PA	79.60%	80.00%
κ	0.734	0.749

Abbreviations: NA, negative agreement; PA, positive agreement; SPT, skin prick test.

Discussion

The objectives of this study were to compare the techniques available for the diagnosis of plant panallergens such as profilin and LTP and to evaluate SPT against in vitro techniques. Our data showed that agreement between SPT, ImmunoCAP, and ISAC microarray with peach LTP Pru p 3 was moderate. Moreover, agreement between SPT with date palm tree profilin (Pho d 2) and ImmunoCAP and microarray with grass pollen profilin (Phl p 12) was also moderate. We are aware that these techniques explore different responses (skin mast cells versus circulating serum sIgE). In addition, for the diagnosis of sensitization to profilin, we compared profilins of different origins, since it is currently not possible to test the same profilin by SPT and by sIgE using commercially available techniques. However, high cross-reactivity between profilins from different sources has been reported [9] and was confirmed in our study between the profilins used in the microarray (ICC=0.976).

We found a level of agreement similar to those reported elsewhere [3,10] when comparing the diagnostic performance of different techniques for assessing sensitization to LTP (even when Advia Centaur was used instead of ImmunoCAP). However, Orovitg et al [10] found that sensitization to profilin determined using SPT with palm tree profilin and sIgE against the same allergen (Pho d 2) determined using Advia Centaur led to better agreement ($\kappa=0.7$) [10] than that observed in our study for SPT against Pho d 2 and sIgE by ImmunoCAP against Phl p 12 ($\kappa=0.507$), as was expected. These results indicate that the differences observed in test results could be related not only to the diagnostic technique itself, but also to the molecule used.

It is noteworthy that the 3 techniques studied (SPT, ImmunoCAP, and ISAC microarray) had different characteristics. On the one hand, SPT is a very accessible test, unlike determination of serum sIgE, which requires special equipment. On the other hand, sIgE can be determined using either ImmunoCAP or microarray without skin lesions or antihistamines, and, unlike SPT, in vitro techniques are also safe in highly sensitized patients. However, sophisticated microarray techniques are more expensive than SPT.

Our study is limited by the lack of a gold standard for selecting the best test for diagnosing sensitization to profilin and LTP. Thus, sensitization to both allergens can be detected in more patients using SPT than in vitro techniques. If the sensitivity of SPT to profilin and LTP is higher than in in vitro techniques, then it should be demonstrated in further studies using a specific gold standard.

In conclusion, component-resolved diagnosis has quantitatively improved the diagnosis of allergy and the management of allergic patients. Skin tests have also been somewhat overtaken by more sophisticated techniques such as component-based allergen microarrays. Nevertheless, the data we report highlight the usefulness of SPT with palm tree profilin and peach LTP for the diagnosis of sensitization to these proteins when in vitro techniques are not available.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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