

Pollen allergy in peach-allergic patients: Sensitization and cross-reactivity to taxonomically unrelated pollens

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Background: Fruit allergy has been attributed to cross-reactive IgE to pollens and has been associated with a particular pollen sensitization.

Objective: The aim of the study was to evaluate sensitization to several taxonomically unrelated pollens in peach- and pollen-allergic patients and to study cross-reactivity between them.

Methods: One hundred sixty-five patients were evaluated: 70 peach-allergic patients together with 95 pollen-allergic patients (control group). Pollen skin tests in duplicate were performed to 5 grasses, 8 trees, and 7 weeds. Cross-reactivity between peach and taxonomically diverse pollens was determined by radioallergosorbent inhibition and Western blot inhibition tests. Experiments were also carried out after preadsorption of the sera with purified natural profilin.

Results: The skin test results revealed that peach-allergic patients frequently reacted to most pollens—grasses, weeds, and trees—even when some of these are not found in our geographic area. There was a statistically significant increase in sensitization frequency to most trees and weeds, with a statistically higher occurrence of asthma (odds ratio 2.98, 95% confidence interval 1.46-6.09). Inhibition test results provided evidence that taxonomically unrelated grasses, weeds, and trees produced various and substantial degrees of inhibition in specific IgE to peach and that the peach extract elicited strong inhibitions to those pollens. Profilin was found to be a relevant cross-reactive antigen in these patients.

Conclusion: The results of this study provide evidence that peach allergy is linked to sensitization to several taxonomically unrelated pollens. This is attributable to the ubiquitous nature of the IgE binding determinants—such as profilins—between peach and taxonomically unrelated pollens. (*J Allergy Clin Immunol* 1999;104:688-94.)

Key words: Fruit allergy, peach, allergy, food, pollen, cross-reactivity, profilin, carbohydrate determinants

The oral allergy syndrome after consumption of fruits of the Rosaceae family in patients with birch pollinosis was described as early as 1948 by Juhlin-Dannfeldt.¹ Fruit allergy has been explained by cross-reactive IgE to

Abbreviations used

CI:	Confidence interval
OR:	Odds ratio
PVDF:	Polivinyldiene difluoride
RAST:	Radioallergosorbent test
TBS:	TRIS-buffered sodium chloride solution

pollen,² and thus allergy to each vegetable has also been reported in conjunction with some particular pollens, for instance, apple to birch,¹ banana-melon to ragweed,³ hazelnut to hazel,⁴ celery to birch-mugwort,⁵ tomato-melon-watermelon to grass,⁶ fennel-celery to birch,⁷ kiwi to grass⁸ or birch,⁹ melon to plantago-grass,¹⁰ etc.

Peach allergy is the most frequent fruit allergy in our geographic area.¹¹ Interestingly, some differences have been reported concerning rosacea fruit allergy in patients from northern Europe and southern Europe; these differences have principally been attributed to birch pollen sensitization in the former and grass pollen sensitization in the latter.^{12,13}

On evaluating peach allergic patients on the basis of our daily clinical experience, we observed that sensitization to several taxonomically unrelated pollens was frequently found, some of these pollens being rare or nonexistent in the Madrid area. The aim of the study has been to evaluate sensitization to several taxonomically unrelated pollens in peach- and pollen-allergic patients and to study whether cross-reactivity exists between peach and pollens.

MATERIAL AND METHODS

Study design

A comparative study was designed to evaluate differences in pollen sensitization frequency by means of skin test reactivity on comparing 2 patient groups, a group of peach-allergic patients and a control group of pollen-allergic patients. Subsequently, inhibition studies (radioallergosorbent [RAST] inhibition and Western blot inhibition) were carried out to demonstrate cross-reactivity.

Patients

One hundred sixty-five patients took part in this study: 70 peach-allergic patients together with a control group of 95 pollen-allergic patients. The peach-allergic patients were selected by criteria of typical symptoms of previous peach allergy reactions, positive prick tests to peach, and a positive peach challenge test.¹⁴

The pollen-allergic control patients were selected by pollen

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allergy criteria and were also evaluated to determine whether they had peach allergy. The patients gave written consent to participation in the study.

Skin tests

Skin tests were carried out in duplicate by the prick-test method and were performed according to guidelines of the European Academy of Allergology and Clinical Immunology.¹⁵ The Alk-Lancet needle (Alk-Lancet, Alk) was used during the study. A result was considered positive when a 3-mm wheal or greater was obtained.

Skin tests to an aeroallergen commercial battery (pollens, mites, fungi, cat, and dog) were performed. The following pollens were tested: 5 grasses (*Lolium*, *Phleum*, *Phragmites*, *Secale*, and *Cynodon*), 7 weeds (*Ambrosia* [ragweed]), *Chenopodium*, *Artemisia* [mugwort], *Taraxacum*, *Salsola*, *Parietaria*, and *Plantago*), and 8 trees (*Betula* [birch]), *Corylus* [hazel nut], *Olea*, *Fraxinus*, *Prunus amygdalus* [almond tree], *Prunus persica* [peach tree], *Populus*, and *Ulmus*).

The prick test with fresh peach was performed in duplicate by the prick-prick method, following the technique described by Dreborg et al.¹⁶

Peach challenge test

The oral challenge tests were carried out with fresh peach, following the method previously described.¹⁷ Briefly, each dose was chewed and then swallowed. The peach doses were gradually increased until a positive response was obtained or until the entire peach was eaten without symptoms. The in vivo tests with fresh peach were carried out from June to October, which is the peach season in Spain.

RAST test and RAST inhibition

Solid-phase antigen (peach peel) was obtained by coupling the extract solution¹⁷ (2 mg/mL) to the 6-mm diameter cyanogen bromide-activated paper discs as described by Ceska and Lunqvist.¹⁸ RAST was performed by the method of Wide et al¹⁹ with Phadezym RAST reagents (Pharmacia Diagnostics AB), and according to the manufacturer's instructions. This chromogenic immunoassay, based on the use of anti-human IgE- β -galactosidase conjugates, was carried out for RAST inhibition studies.

The RAST inhibition technique was performed as previously reported.²⁰ Fifty microliters of a serum pool from patients sensitive to peach extract plus 50 μ L of 3 (10-fold) dilutions of allergenic extracts (inhibitors in liquid phase) was added to solid-phase peach peel allergen. The following allergenic extracts were used as inhibitors: *Corylus*, *P amygdalus*, *Betula*, *Phleum*, and *Artemisia*.

Western blot

SDS-PAGE was carried out according to the method of Laemmli²¹: 12.5% and 3% polyacrylamide concentrations were used for separating and stacking gels, respectively. Proteins dissolved in 0.125 mol/L hydrochloric acid-TRIS, pH 6.8, were dissociated with 0.1% SDS and 5% β -mercaptoethanol by treatment at 100°C for 5 minutes, and 20 μ g of protein of mugwort (*Artemisia*), birch (*Betula*), *Corylus*, and *P amygdalus* was applied per lane.

After electrophoresis, the separated protein bands were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore), essentially according to Towbin et al.²² Antigen-antibody-binding was identified as described by Shen et al.²³ In brief, immunochemical staining was performed by first blocking the Immobilon membrane with 90% defatted dry milk in TRIS-buffered sodium chloride solution (TBS) (1 hour at 37°C). After 3 washes with TBS, blots were incubated

with 2.5 mL of undiluted human serum (16 hours at 4°C). The blots were washed 3 times with TBS and incubated with 2.5 mL of 1:50 peroxidase-conjugated rabbit immunoglobulins to human ϵ -chains (Dako, Glostrup, Denmark). Bound peroxidase was detected by immersion in an enhanced chemiluminescence solution (ECL kit, Amersham). The specific IgE-binding bands were identified after exposure to a Kodak X-omat film for 60 seconds. The amount of the specific IgE bound to each strip was estimated in the Bio-image analyzer.

Western blot inhibition

Aliquots of the undiluted serum pool and individual sera from peach-allergic patients showing RAST values to peach more than or equal to class 3 were incubated for 10 minutes with equal volumes of the peach peel extract (100 μ g/mL protein) or purified natural profilin (10 μ g/mL protein). Mixtures were then centrifuged and supernatants were incubated with pollen-blotted PVDF strips, followed by immunodetection as described in the Western blot section. The sera from 10 nonatopic subjects with no history of fruit allergy or pollinosis were pooled and used as negative controls in all immunochemical techniques.

Profilin was extracted from sunflower pollen, purified, and characterized according to the methods previously described.²⁴

Specific IgE to recombinant Bet v 1 and Bet v 2

Specific IgE to recombinant allergens Bet v 1 and Bet v 2 (profilin) was stimulated by the Pharmacia CAP system.

Statistical analysis

Qualitative variables were described by absolute (n) and relative (percentage) frequencies. To evaluate the association between each pollen and peach allergy, individual 2×2 contingency tables were constructed. The magnitude of the association was estimated by the odds ratio (OR) and 95% confidence intervals (95% CI). The statistical significance was evaluated by chi-square test. All *P* values were 2-tailed.

RESULTS

General characteristics

Peach allergy group. Seventy peach-allergic patients were evaluated, 34 (49%) male and 36 (51%) female. The mean age was 20.3 ± 8.18 years, varying from 4 to 43 years. The symptoms of peach allergy were oral allergy syndrome 82.8%, contact urticaria 53%, and systemic symptoms 25.7%.

Fifty-seven of the 70 (81%) peach-allergic patients had allergic respiratory disease and were allergic to pollen. Fifteen of these pollen-sensitive patients (26.7%) had rhinitis and 42 (73.3%) had rhinitis-asthma. When the first allergy—either peach or pollen—reported by the pollen-sensitive patients allergic to peach was evaluated, it was observed that pollen symptoms were the first to appear in 27 of 55 patients (49.09%), peach in 19 of 55 patients (35.55%), and symptoms to peach and pollen began in the same year in 9 of 55 (16.36%) patients. Two patients were unable to remember this piece of data.

Pollen allergy control group. Fifty-four (56%) of the pollen-allergic patients in the control group were males and 42 (44%) females. All had respiratory symptoms, 49 (52%) rhinitis and 46 (48%) rhinitis-asthma; thus the OR

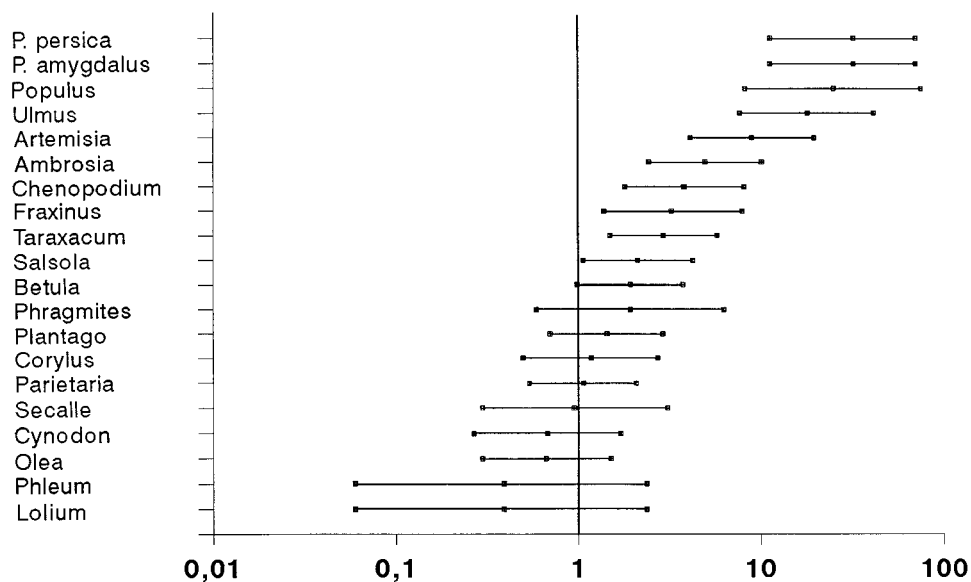


FIG 1. OR and 95% CI of association between each pollen and peach allergy. There was statistical significance ($P < .05$) when 1.0 was not included in CI.

TABLE I. Sensitization frequency to grass, tree, and weed pollens in pollen-allergic patients from peach allergy group and pollen allergy control group

	Peach and pollen group (n = 57)		Pollen control group (n = 95)		Statistical significance
	n	%	n	%	
<i>Cynodon</i>	47	82.5	83	87.4	NS
<i>Lolium</i>	54	94.7	93	97.9	NS
<i>Phleum</i>	54	94.7	93	97.9	NS
<i>Phragmites</i>	53	93.0	83	87.4	NS
<i>Secale</i>	52	91.2	87	91.6	NS
<i>Betula</i>	35	61.4	43	45.3	$P < .05$
<i>Corylus</i>	47	82.5	76	80.0	NS
<i>Fraxinus</i>	49	86.0	62	65.3	$P < .05$
<i>Olea</i>	43	75.4	78	82.1	NS
<i>Populus</i>	53	93.0	33	34.7	$P < .001$
<i>P. persica</i>	49	86.0	17	17.9	$P < .001$
<i>P. amygdalus</i>	49	86.0	17	17.9	$P < .001$
<i>Ulmus</i>	46	80.7	18	18.9	$P < .001$
<i>Ambrosia</i>	35	61.4	23	24.2	$P < .001$
<i>Artemisia</i>	45	78.9	28	29.5	$P < .001$
<i>Chenopodium</i>	45	78.9	47	49.5	$P < .001$
<i>Parietaria</i>	23	40.3	37	38.9	NS
<i>Plantago</i>	41	71.9	61	64.2	NS
<i>Salsola</i>	40	70.2	50	52.6	$P < .05$
<i>Taraxacum</i>	36	63.2	35	36.8	$P < .01$

NS, Not significant.

for asthma in comparison with the peach allergy group was 2.98 (95% CI 1.46-6.09). Twenty-five (26.3%) patients in the pollen allergy control group had positive prick-prick tests, and only 12 (12.6%) reported peach allergy symptoms as well as having a positive peach provocation test.

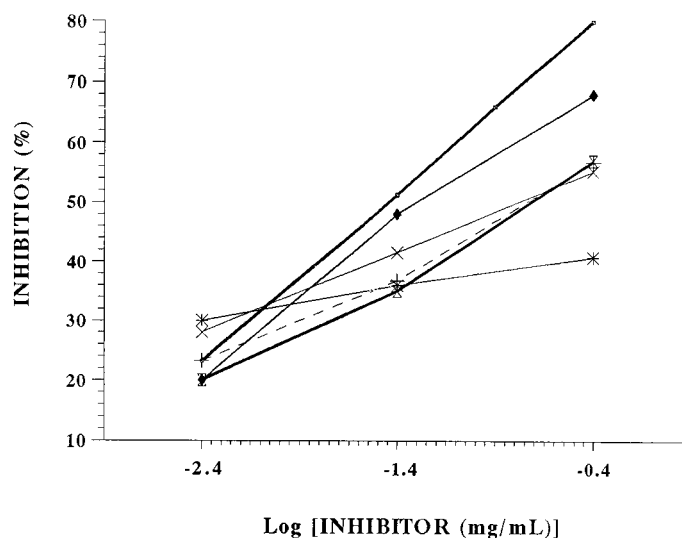
Pollen skin tests

The results of pollen skin tests in pollen allergic control patients were similar to those that have been previously reported in the central region of Spain (Madrid area); therefore the higher sensitization number was caused by grass pollens represented by *Lolium* and *Phleum* (97.9%), followed by tree pollens represented by *Olea* (82.1%) and weed pollens represented by *Plantago* (64.2%).

The results of pollen skin tests in the pollen-allergic patients from the peach allergy group and the pollen control group are shown in Table I. The OR and 95% CI for each pollen are shown in Fig 1. We should first emphasize the high sensitization frequency to all pollens tested in the peach allergy group; the lowest sensitization frequency was more than 60%. The increase in pollen sensitization frequency was statistically significant for most of the tree and weed pollens, with the exception of *Corylus*, *Olea*, *Plantago*, and *Parietaria*. This increase in sensitization frequency had its highest OR in pollens such as *P. persica* (32.2), *P. amygdalus* (32.2), *Populus* (24.9), and *Ulmus* (17.9), increasing from less than 20% of the patients to more than 80%.

RAST inhibition

Inhibitions of IgE binding to peach by pollens are represented in Fig 2. The ubiquitous nature of the IgE-binding determinants was demonstrated by RAST inhibition tests with peach allergen disks and extract from taxonomically unrelated pollens, leading to significant inhibitions among all these allergen sources. All the *Phleum*, *Corylus*, *P. persica*, *Betula*, and *Artemisia* extracts elicited significant degrees of inhibition in the binding specific IgE to peach, demonstrating that there was cross-reactivity between taxonomically unrelated pollen and peach



	—●— Peach	—+— Corylus	-*- Prunus	-*- Betula	—◆— Artemisia	-*- Phleum
Ag50	0.03	0.16	---	0.14	0.06	0.18
MI	80%	57%	41%	55%	68%	57%

FIG 2. RAST inhibition curves with use of peach-peel extract in solid phase. Ag50, Inhibitor (pollen) concentration eliciting 50% of RAST inhibition (in milligrams per milliliter); MI, maximal inhibition.

extracts. The *Artemisia* extract produced the maximal inhibition of specific IgE to peach (68%).

Western blot and Western blot inhibition

Western blot inhibition experiments were carried out in 5 individual sera as well as in a serum pool composed of these sera to investigate the presence of common IgE epitopes in taxonomically unrelated pollens shared by peach. The sera were preadsorbed with peach extract or natural profilin. Inhibition of IgE binding to pollens (*Artemisia*, *Betula*, *Corylus*, and *P persica*) by peach extract or natural profilin is shown in Fig 3. The molecular weights of each single IgE-binding band in pollen extracts, as well as the percentage of inhibition elicited by preadsorption of the serum pool with peach or natural profilin extracts, are shown in Table II.

In our study preincubation of sera with peach extract led to an important inhibition of IgE binding to PVDF-blotted pollen extracts, indicating that a high degree of cross-reactivity existed between taxonomically diverse pollens and peach. IgE reactivity to the 15.8-kd band—corresponding to profilin—disappeared when the sera were inhibited with natural sunflower profilin. Profilin was found to be an antigen shared by peach and taxonomically diverse pollens, producing cross-reactivity in our patients. No IgE reactivity to the nonallergic control sera was observed.

Artemisia extract inhibition. Twelve IgE-binding bands were identified in the *Artemisia* extract with the serum pool, within a range of 15.8 and 106 kd. The peach extract was

capable of producing a complete inhibition in most of the bands, for example, 15.8, 17.4, 19, 24, 26, 41, 45, 70, 85, and 106 (extrapolated value) kd. The 35-doublet and 30-kd bands continued unaltered after preadsorption of the serum with peach extract. Preadsorption of the sera with profilin elicited total inhibition of the 17.4- and 85-kd bands and partial inhibition in the 15.8-, 19.2-, and 106-kd bands.

Betula extract inhibition. Ten IgE-binding bands were detected in the *Betula* extract within a range of 16 to 96 kd. Peach extract was capable of producing a complete inhibition of all bands, except 3 of them, which were partially inhibited such as 27 kd (80%), 43.7 kd (25%) and 67 kd (58%). The possibility that the 18.9-kd bands corresponded to Bet v1 was ruled out because specific IgE to Bet v1 was not detected in any of the sera (Table III).

Corylus extract inhibition. Ten IgE-binding bands were detected in the *Corylus* extract with a range of 15.8 to 131.8 kd. The peach extract was capable of producing an almost complete inhibition of 4 bands, 3 in the lowest molecular weight (15.8, 18.6, and 22.3 kd), and 1 in the 132-kd band. Pre-adsorption of the sera with profilin was only capable of eliciting partial inhibition of the bands inhibited by peach extract.

P persica extract inhibition. Twelve IgE-binding bands were detected in *P persica* extract with a range of 16.8 to 142 kd. Preincubation of the sera with profilin elicited a considerable inhibition of the two 18.7- and 16.8-kd bands. The 37.9-, 31.6-, and 29.7-kd bands were also strongly inhibited by profilin; the possibility that

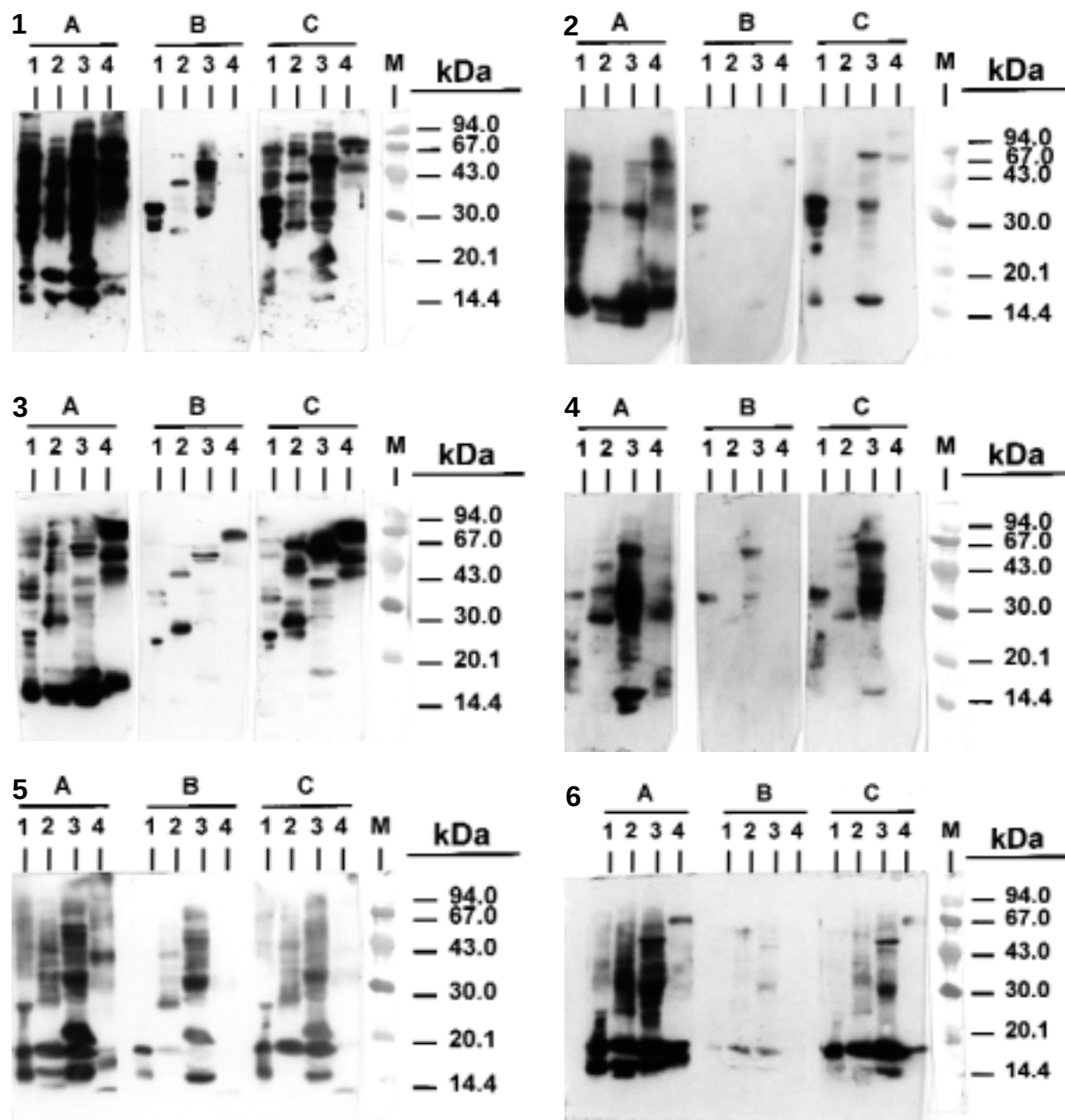


FIG 3. Western blot inhibition of pollens. **3.1,** Western blot inhibition of pollens with serum pool. **3.2 to 3.6,** Western blot inhibition of pollens with individual patient sera. Lane 1, *Artemisia* pollen; lane 2, *Betula* pollen; lane 3, *Corylus* pollen; lane 4, *P. persica* pollen. M, Molecular mass markers; A, noninhibited Western blot; B, Western blot inhibitions with preadsorption of sera with peach extract. C, Western blot inhibition with preadsorption of sera with profilin.

these latter bands correspond to profilin aggregates cannot be ruled out because polymerization of profilins through disulfide bonds has recently been reported.²⁵ Preadsorption of the sera with peach extract was capable of eliciting inhibition of the bands inhibited by profilin, as well as inhibiting others with greater molecular weights, such as 53.2 kD, 60.7 kD, 88.5 kD, and greater than 94 kD. This may indicate that there are common epitopes shared by peach and pollens differing from profilin.

DISCUSSION

This study was carried out to evaluate a clinical event arising in our daily medical practice that is not in agreement with the data that have been reported to date. The results of this study have demonstrated the existence of sensitization to taxonomically unrelated pollens in peach-allergic patients. We attribute this to the ubiquitous nature of the IgE-binding determinants between peach and taxonomically unrelated pollens.

TABLE II. Pollen IgE binding bands and percent inhibition caused by peach and profilin extracts

Blotting	Noninhibited blot band (kd)	Peach-inhibited blot (%)	Profilin-inhibited blot (%)
<i>Artemisia vulgaris</i>	105.9	100	76
	85.0	100	100
	69.7	100	0
	45.1	100	0
	41.1	100	0
	34.6	0	0
	30.2	0	0
	26.5	100	0
	23.6	100	0
	19.2	100	15
	17.4	100	100
	15.8	100	30
<i>Betula alba</i>	95.8	100	64
	78.5	100	57
	67.0	58	0
	55.0	100	100
	43.7	25	5
	37.9	92	73
	34.0	92	45
	27.7	80	42
	18.9	100	96
	16.0	100	95
<i>Corylus avellanus</i>	131.8	100	85
	97.8	25	32
	61.7	0	0
	45.9	0	0
	38.5	0	0
	33.4	0	0
	27.5	0	0
	22.3	100	0
	18.6	100	39
	15.8	97	57
<i>P amygdalus</i>	142.8	100	100
	88.5	99	57
	60.7	95	35
	53.2	100	0
	37.9	100	85
	31.6	100	100
	29.7	100	100
	18.7	100	100
	16.8	100	100

Grasses and trees make up the major pollinating plants in the Madrid area. It is important to remember that in Madrid—in contrast to other geographic areas—some major allergenic weeds (*Ambrosia* or *Parietaria*) and trees (*Betula*) are rarely found or are totally absent. Pollen sensitization frequency in pollen-allergic patients in our area has previously been reported by Subiza et al,²⁶ who were in agreement with the pollen skin test results that we observed in our pollen-allergic control group.

Pollen allergy has frequently been found among

TABLE III. Specific IgE to Bet v 1 and Bet v 2

Patient No.	Bet v 1	Bet v 2
1 (serum pool)	ND	ND
2	<0.35	15.20
3	<0.35	5.19
4	<0.35	<0.35
5	<0.35	9.12
6	<0.35	6.60

ND, Not done.

Patient number corresponds to patient number in Fig 3.

peach-allergic patients (odds 4:1); therefore pollinosis might be considered a risk factor in the development of peach allergy. Despite this fact, the results of this study provide evidence that peach allergy is linked to a special pollen sensitization. The peach-allergic patients in our study frequently reacted to several pollens—grasses, weeds, and trees—even when some of these species have never been encountered in our area. There was a statistically significant increase in sensitization frequency to most taxonomically unrelated trees and weeds, reflecting a completely different pollen sensitization frequency with a statistically higher occurrence of asthma (OR 2.98). The skin test results suggest that the increase in sensitization frequency may be the result of cross-reactivity to some ubiquitous antigenic determinant shared by both peach and pollens. The results of inhibition tests—both RAST inhibition and Western blot inhibition—confirm this. Taxonomically unrelated grasses, weeds, and trees were capable of producing substantial inhibition of specific IgE to peach. The peach extract, in turn, produced strong inhibition in the respective pollens.

Allergenic cross-reactivity among pollens is well known,²⁷ and such relationships have generally been believed to occur between taxonomically closely related species. Attention has been given to the hypothesis that the presence of reactivity to an individual pollen may not necessarily have been produced because of contact with that pollen or even with a taxonomically closely related species²⁸; in this context cross-reactive antigens have been reported, such as profilin,²⁹ Art v 1,³⁰ cross-reactive carbohydrate determinants,^{31,32} Ole e 3,³³ etc.

Our results point to the hypothesis that fruit allergy (peach) may be a risk factor in this matter. Western blot results have highlighted the fact that profilin is a common antigen shared by pollens and peach; this may explain in part the sensitization to taxonomically diverse pollens found in peach-allergic patients. Profilin—a family of cytoskeleton proteins—has been demonstrated to be an allergenically cross-reactive family of proteins found in both pollens and vegetable foods.²⁹ The clinical role of profilins is not as clear as that of pathogenesis-related proteins.³⁴ It has been reported^{12,34,35} that profilins are strong sensitizers, but the weak correlation between positive skin tests with profilin and allergic reactions to vegetables suggest that profilins do not often cause such frequent clinical symptoms. Our results suggest a possible

role of profilin sensitization in the higher prevalence of asthma observed in peach-allergic patients; this should be demonstrated in future studies.

Although in our study profilin was the most evident cross-reactive allergen shared by taxonomically diverse pollens and peach, preincubation of the sera with peach extract led to a higher inhibition of the IgE binding to PVDF-blotted pollen extracts than with profilin, indicating that other IgE epitopes may also be implicated.

We believe that the data reported in this article are relevant for daily medical practice and we demonstrated that peach allergy is not associated with sensitization to any particular pollen, as has been reported with other fruit allergies. It is our belief that sensitization to taxonomically diverse pollens is instead linked to peach allergy; this may be a risk factor in sensitization to taxonomically diverse pollens, as expressed by a higher asthma frequency.

We would therefore suggest that patients showing sensitization to most taxonomically unrelated pollens be questioned about fruit allergy and that these findings be kept in mind when pollen allergy in fruit- (peach) allergic patients is evaluated. Physicians should also be careful not to diagnose cross-reactive pollen sensitization as relevant because this may have important consequences in prescribing specific pollen immunotherapy.

We do not know to what extent our findings can be extrapolated to other fruit allergies or to other geographic areas; this can only be evaluated in future studies.

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