

Plant Food Allergy in Patients with Pollinosis from the Mediterranean Area

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Key Words

Food allergy · Food hypersensitivity · Pollinosis ·
Plant-food allergy · Oral allergy syndrome

Abstract

Background: Patients with pollinosis develop symptoms after intake of plant food more often than the general population. In order to study the prevalence of the presentation of allergic symptoms to plant foods in pollinosis, we selected a representative sample of the population from our Mediterranean area. **Methods:** All patients completed a questionnaire, provided a blood sample and underwent a battery of skin and other complementary tests (prick-prick, oral challenge test) when necessary. The pollen counts were obtained from the Elche pollen station. In addition, sera from a subgroup of patients were checked with an allergen molecule panel on an Advia Centaur XP platform. **Results:** Of the final sample (n = 233), 39.9% of the patients with pollinosis were sensitized and 30.9% had clinical allergy to at least one of the plant foods studied. Regression analysis showed that age and sensitization to the extracts of *Platanus acerifolia* and *Artemisia vulgaris* were the most important variables for discriminating between groups. Patients with pollinosis at a risk of allergy to plant foods had significantly higher Pru p 3 values [odds ratio (OR) 3.3; 95% confidence interval (95% CI) 2.3–4.8], and the value increased according to the number of plant food sensitizations. **Conclusion:** Plant food allergy is

more common in patients with pollinosis than in the general population. The use of the London plain tree (*P. acerifolia*) and mugwort (*A. vulgaris*) in the skin tests may help identify such patients in our Mediterranean area, but determination of rPru p 3 could also be very useful in patients suspected of having plant food allergy.

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Introduction

Between 30 and 60% of pollen-allergic patients in Europe are estimated to be allergic to plant foods [1, 2]. After the intake of plant foods, these patients experience adverse reactions (urticaria, angioedema, rhinoconjunctivitis, asthma, anaphylaxis and oral allergy syndrome) more frequently than the rest of the general population [3]. The magnitude of the problem is aggravated by the very high – and apparently increasing [4] – prevalence of pollen allergy in developed countries (15–25%).

In Spain and other European countries, the main cause of allergic reactions in adults is plant food [5]. Allergy to food also impacts on the quality of life of affected patients and it is an important factor in the social and economic burden in developed countries [6–8].

Isolation, characterization and cloning of the main allergens involved in cross-reactivity between pollen and food enabled the study of the homology between pollens

and plant foods [9, 10]. In addition, this advance has led to the introduction of purified allergens as a diagnostic aid, defined as 'component-resolved diagnostics' [11]. The panels of allergen extracts were complemented with the recognition and individual quantification of the main molecular allergens through component-resolved diagnosis in order to improve the response to a whole extract.

The environmental factors to which the population is exposed are also of great interest. The main dietary habits and pollinoses in each geographical area influence certain allergy patterns to pollen and plant food. In northern and central Europe, allergic reactions to plant foods are associated with birch pollen allergens, Bet v 1 homologs and profilins. In the Spanish Mediterranean area, however, lipid transfer proteins (LTP) are more important. This area is characterized by low rainfall, warm temperatures, and high levels of olive, *Chenopodiaceae*, grass and *Parietaria* pollens, similar to other Mediterranean countries. Nevertheless, not all patients develop the same pattern of sensitization, nor do all patients necessarily recognize the same allergens, and there is even great variation in symptoms from one patient to another [12, 13].

Our aim was to undertake a representative study of the clinical characteristics, associated factors and molecular profile of pollinosis with and without a risk of plant food allergy in our Mediterranean area.

Methods

We undertook a cross-sectional, observational, descriptive study.

Study Population

We studied a sample of 396 consecutive patients who attended the Allergy Office of the Elche University Hospital, Alicante, Spain, due to pollinosis. The patients were recruited over 18 months. The inclusion criteria were a diagnosis of pollen allergy with clinical symptoms (rhinoconjunctivitis or asthma) and skin test sensitization, age older than 14 years, and provision of signed informed consent. The exclusion criteria were pregnancy or breast-feeding, skin diseases, psychological or psychiatric disorders, contraindication for adrenaline, drug or alcohol abuse or inability to complete the established protocol. The study was approved by the local ethics committee. All patients provided signed informed consent.

Diagnostic Procedures

The patients all completed a questionnaire, provided a blood sample and underwent a battery of skin tests on the first day. The data were recorded in a data base.

Questionnaire: This gathered epidemiological data (age, address and occupation), and data on pollen allergy (rhinoconjunctivitis and asthma), food allergy (exposure and symptoms), latex allergy and other allergy-related data.

Skin-Prick Test: This was performed following the method proposed by the European Academy of Allergology and Clinical Immunology [14, 15]. We used commercial extracts (ALK-Abelló, Madrid, Spain) of the 10 most common pollens in Spain (olive, grass, birch, cypress, London plane, mugwort, pellitory, London plantain, Russian thistle and date palm) as well as 15 plant food extracts that are most frequently involved in allergic reactions in our area (peach, mustard, almond, hazelnut, peanut, chestnut, walnut, sunflower seeds, lentils, soy, melon, kiwi, apple, tomato and banana) and another 5 common aeroallergens in the study area (mites, cat dander, dog dander, *Alternaria* and latex). All the skin test series included histamine dihydrochloride (10 mg/ml) and physiological saline as a positive and negative control, respectively. The tests were carried out in duplicate. The response was evaluated by measuring the size of the wheal, using the equation: $(D + d)/2$, where D is the maximum diameter and d the perpendicular diameter. The tests were considered positive if the wheal area exceeded the diameter of the negative control by 3 mm [16].

Patients with negative skin tests in the presence of symptoms were requested to attend at a later date to undergo complementary tests of specific IgE using UniCap 100 (Phadia, Upsala, Sweden) according to manufacturer's instructions, prick-prick tests with culprit foods were done according to the EAACI position paper [17] and open challenge tests (peach, melon, kiwi, tomato and banana), following the diagnostic algorithm of the Food Adverse Reaction Committee of the Spanish Society of Allergology and Clinical Immunology [18]. Patients suffering from severe systemic reactions to plant foods, as well as patients with typical, immediate, repeated and unequivocal reactions who had a positive skin test did not undergo an open challenge test to diagnose plant food allergy [18].

The patients were classified in two groups as a function of the results. Group 1 (PP), comprising patients with pollinosis, consisting of those patients who exhibited no sensitization or clinical symptoms to any of the plant foods studied, and group 2, comprising patients with pollinosis and a risk of food allergy (PPR), comprising those patients with a risk of plant food allergy, i.e. patients with food sensitization but without clear clinical symptoms and patients with sensitization and clinical symptoms to at least one of the plant foods studied. This second group included the two types of patients together (both with and without plant food symptoms) because we considered them to be 'at risk' of plant food allergy with the presence of IgE to a particular allergen.

Allergen Molecular Panel

After classifying the patients into the two groups, we studied a molecular allergen panel in a subgroup of 112 patients, including most of the patients with a risk of food allergy (66 of 72 patients) from the PPR group and a random sample of 46 patients from the PP group. In this subgroup of patients, we measured recombinant and native allergens in order to improve the diagnosis of patients allergic to pollen who also reported food allergies. The method used was the Advia Centaur XP platform (Siemens Medical Health Care Diagnosis Europe Ltd., Saint-Vulbas, France) [19]. Results above 0.35 KU/l were considered positive. The allergens were isolated by the Vegetalia Network [20]. The individual aller-

gens included in the diagnostic panel were (n = native antigen; r = recombinant antigen; rNtD = recombinant antigen amino terminus; rCtD = recombinant antigen carboxy terminus): *Olea europaea*: nOle e 1 and Ole e 9, a mix of rNtD Ole e 9 and rCtD Ole e 9; *Artemisia vulgaris*: nArt v 1; *Phleum pratense*: nPhl p 1 and nPhl p 5; *Cynodon dactylon*: nCyn d 1; *Plantago lanceolata*: nPla l 1; *Parietaria judaica*: nPar j 1; *Cupressus sempervirens*: nCup s 1; *Salvia kali*: nSal k 1; *Chenopodium album*: rChe a 1, rChe a 2 and rChe a 3; *Platanus acerifolia*: rPla a 1 and nPla a 2 (BIAL-Aristegui, Bilbao, Spain). As pan-allergens we used: LTP: rPru p 3 (peach) and a mixture of profilins: rCuc m 2 (melon), rChe a 2 from the pollen of *C. album* and rMal d 4 (apple). We also included the purified proteins: lentil: nLen c 1; chitinases: mixture of nPers a 1 (avocado) and rCas s 5 (chestnut); proteins from the PR-10 family: nBet v 1 of *Betula verrucosa*; proteins from the 2S albumin family: nSin a 1 (mustard), and peroxidase as a CCD marker (Sigma-Aldrich Co., St. Louis, Mo., USA) [20].

Pollen Count

The pollen counts during the study period were obtained from the Elche pollen station (in operation since 1993) and a member of the Aerobiology Committee of the Spanish Society of Allergy and Clinical Immunology [21]. The results are expressed as total annual pollen count (pollen grains/m³).

Statistical Analysis

The statistical analyses were done with the SPSS software (SPSS Inc., Chicago, Ill., USA). The sample size was calculated from a previous study on 50 patients with pollinosis, which gave a 23% frequency of allergy to plant foods. The 95% confidence level with a confidence interval of 11.66 showed that the sample size necessary for a representative study in the study population (360,000 inhabitants), assuming a 20% loss was a minimum of 147 pollinosis patients.

The statistical analysis included a descriptive analysis of the variables. Normal quantitative variables were compared with the Student t test and ANOVA for comparison of the groups. The intermediate bivariate analysis of the qualitative variables of interest was done with frequency distribution tables. The comparative study of the two-by-two percentages was done by the χ^2 test.

To compare and determine the significant factors for sensitization to plant foods in patients with pollinosis (PP vs. PPR), we used a multivariate binary logistic regression analysis.

Results

Study Population

Figure 1 shows the inclusion tree of the 396 consecutive patients studied; we excluded 127 patients who failed the inclusion criteria and 30 patients who had at least one of the exclusion criteria. A further 6 patients were excluded during the study as they did not follow the established protocol. All the patients completed the questionnaire, provided a blood sample and underwent the skin test battery. In patients whose results were doubtful, we performed a total of 193 specific IgE tests (UniCap), 25 prick-

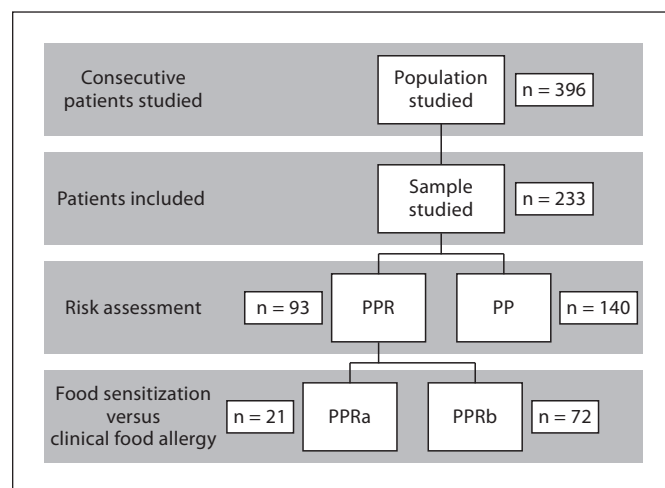


Fig. 1. Diagram of the patients included in the study.

prick tests with the foods involved, and 10 oral open challenge tests to: peach (1 positive of 4), melon (1 positive of 3), kiwi (1 positive of 1), tomato (1 negative) and banana (1 negative). The final population sample, therefore, comprised 233 adult patients (female/male ratio 156:77; median age, 35.2 years, range 14.6–76.6 years). The sample was representative of the study population.

After classifying the patients in the different groups (fig. 1), the PP group included 140 patients (60.1%) and the PPR group, 93 patients (39.9%). The PPR group was composed of 21 patients (9.1%) with food sensitization but with no clinical symptoms (PPRa group) and 72 patients (30.9%) with sensitization and clinical symptoms to at least one of the plant foods studied (PPRb group).

The profile of the study sample (table 1) was a 35-year-old woman, working in the service sector and with an urban dwelling. Most patients had a family history of allergy and reported rhinitis.

Significant differences were noted between the study groups in the demographic characteristics of age and occupation. The PPR patients with (a risk of) plant food allergy were significantly ($p < 0.01$) younger than the PP patients (just patients with pollinosis). A significantly higher proportion of PPR patients ($p < 0.01$) also had a personal history of atopy (asthma or atopic dermatitis or both). Finally, the clinical symptoms of asthma were significantly ($p < 0.05$) more perennial in the PPR patients as compared with the PP patients, who more often displayed the seasonal form. This fact confirms the difference found in the nonseasonality of asthma in patients with pollen-food allergy, as previously described in Spain [19].

Table 1. Sample characteristics

Group/variable	Sample n	PP group n	PPR group n
Patients	233	140 (60.1)	93 (39.9)
Men	77 (33)	42 (29.8)	35 (37.6)
Mean age, years ¹	35.2	40.6	28.2
95% CI		37.4–43.8	25.8–30.7
<i>Occupation</i>			
Primary sector	1 (0.4)	1 (0.7)	0
Secondary sector	42 (18)	29 (20.6)	13 (14.0)
Tertiary sector	77 (33)	43 (30.5)	33 (35.5)
Student ¹	41 (17.6)	19 (13.5)	22 (23.7)
No work	35 (15)	29 (20.6)	6 (6.5)
<i>Post code</i>			
Urban	183 (85.6)	120 (80.5)	63 (87.5)
Rural	30 (14.1)	21 (19.5)	9 (12.5)
<i>History</i>			
<i>Family</i>			
Rhinitis	92 (39.5)	56 (40.0)	32 (34.4)
Asthma	41 (17.6)	19 (13.6)	22 (23.7)
Dermatitis	15 (6.4)	13 (9.3)	2 (2.2)
Food allergy	5 (2.2)	1 (0.7)	2 (2.2)
<i>Personal</i>			
Rhinitis	51 (21.9)	19 (13.6)	33 (35.5)
Asthma	35 (15)	17 (12.1)	18 (19.4)
Dermatitis	24 (10.3)	10 (7.1)	14 (15.1)
<i>Symptoms</i>			
<i>Rhinitis</i>			
Seasonal	231 (99.1)	141 (100)	88 (94.6)
Perennial	73 (31.2)	39 (27.7)	34 (36.6)
<i>Asthma</i>			
Seasonal	105 (45.1)	66 (46.8)	39 (41.9)
Perennial ¹	40 (38.1)	20 (14.2)	20 (21.5)

Figures in parentheses are percentages.

The table shows the distribution of the demographic and comorbidity characteristics of the whole sample and for each of the study groups.

¹ Significant differences between groups.

Pollen Sensitization

The distribution of pollen sensitization detected by skin testing is shown in table 2. Pollinoses to *Olea* spp. (73.8%) and *Salsola* spp. (73.0%) were the most usual in our area, followed by Gramineae (43.8%), *Plantago* (37.8%), *Artemisia* (33%), *Platanus* (25.8%), *Parietaria* spp. (22.7%), *Phoenix* spp. (20.4%), *Cupressus* spp. (16.7%) and *Betula* (11.9%). The pollen distribution frequency varied depending on the study group.

Table 2. Sensitization to pollens detected by skin testing

Allergen	Sample	PP group	PPR group
<i>Olea</i> spp.	172 (73.8)	107 (75.9)	65 (70.1)
<i>Betula</i> spp. ¹	26 (11.9)	7 (5.0)	19 (20.4)
<i>Cupressus</i> spp.	39 (16.7)	21 (14.9)	18 (19.4)
Gramineae	102 (43.8)	57 (40.4)	45 (48.4)
<i>Salsola</i> spp.	170 (73.0)	100 (70.9)	70 (75.3)
<i>Plantago</i> spp. ¹	88 (37.8)	43 (30.5)	45 (48.4)
<i>Parietaria</i> spp.	53 (22.7)	26 (18.4)	25 (26.9)
<i>Artemisia</i> spp. ¹	77 (33.0)	28 (19.9)	49 (52.7)
<i>Platanus</i> spp. ¹	60 (25.8)	20 (14.2)	40 (43.0)
<i>Phoenix</i> spp. ¹	46 (20.4)	22 (15.7)	24 (25.8)

Figures in parentheses are percentages.

The table shows the pollinoses found in the sample and classified according to group. The most common pollinoses in all groups were caused by *Olea* and *Salsola* though their distribution by group varied between the two predominating pollens.

¹ Significant differences between the study groups ($p < 0.05$).

Figure 2 shows the skin sensitizations and air concentrations of the most common pollens. Some pollens (*Betula* spp., *Plantago* spp., *Artemisia* spp. and *Platanus* spp.) with minimal or nonexistent ambient concentrations were associated with a high number of sensitizations. Figure 3 shows the distribution of pollen sensitization in the two study groups. Pollens not present in the air were significantly more frequent in the PPR group (patients sensitized or allergic to plant foods).

Food Sensitization and Symptoms

Table 3 shows the sensitizations and reported clinical symptoms in relation to the plant foods studied. The foods that caused most problems were peach and seed-derived allergens, like sunflower seeds, peanut, walnut, hazelnut and chestnut. Some foods, like lentils and soy, produced sensitization but only rarely clinical symptoms.

Molecular Characterization

Table 4 shows the molecular characterization results of 112 sera from the subgroups of patients studied, comprising most of the PPRb and a random sample of the PP of comparable age (33 vs. 31 years; $p > 0.05$). The allergens Ole e 1 and Sal k 1 were the most prevalent allergens in this subgroup. Of note was the low frequency of profilins (5.4%). Significant differences ($p < 0.01$) between the groups were only found for Pru p 3; Pru p 3 attained a sensitivity of 68% and specificity of 98% to detect Pru p 3

Fig. 2. Comparison of sensitizations and outdoor pollen concentrations. Concentration of ambient pollens (green) and percent sensitizations among the patients (purple). Pollens not present in the air were associated with high percentages of sensitization.

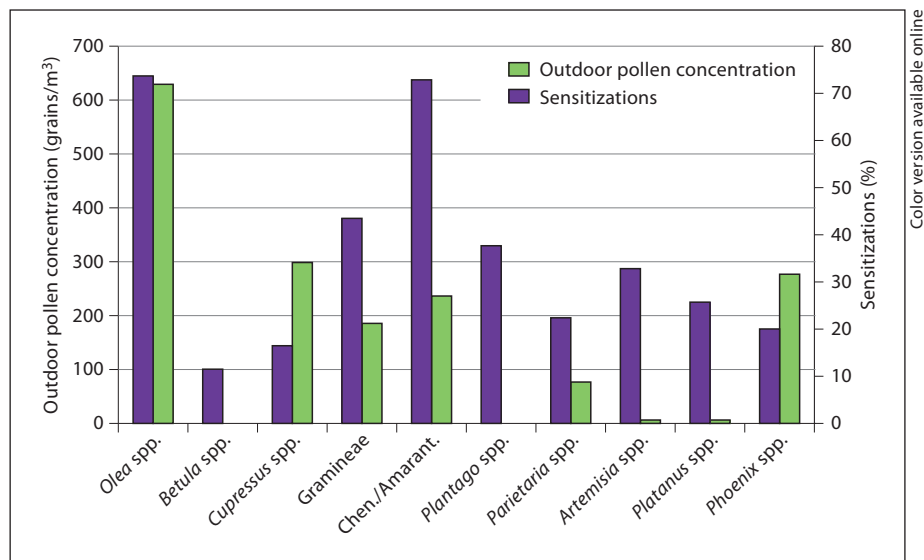
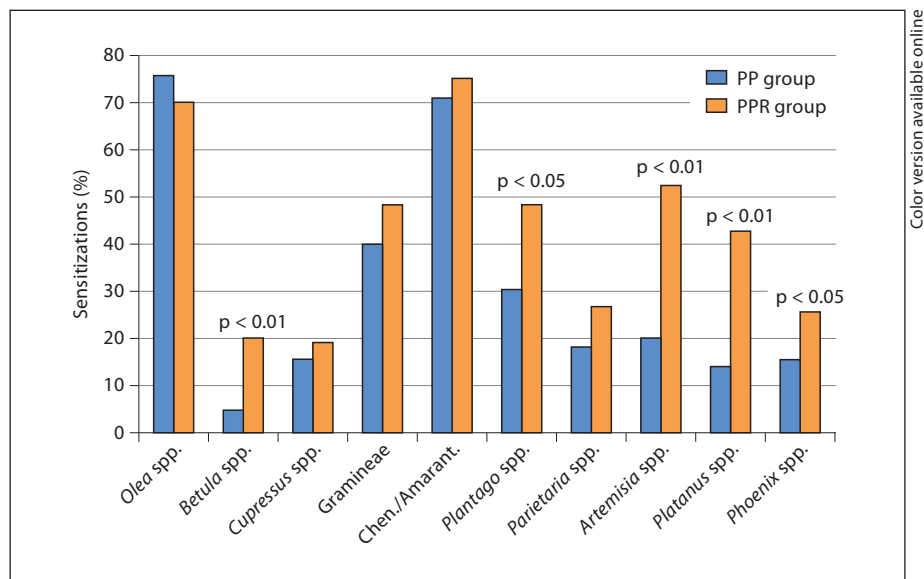


Fig. 3. Pollen sensitization in the PP and PPR groups. Percent sensitization to pollens in the study groups. Significant differences between the groups were found for birch, weed, mugwort, plane and date palm.



levels above 0.35 KU/l [odds ratio (OR) 3.3; 95% confidence interval (95% CI) 2.3–4.8].

Those patients with fewer than 3 sensitizations to plant-derived food ($n = 61$) had significantly lower levels ($p = 0.003$) of rPrU p 3 (0.8 KU/l) than the patients ($n = 51$) with 3 or more sensitizations (20.6 KU/l).

Correlation of the skin tests with the main allergen determinants was only found ($p < 0.01$) between the extract and the main allergen in olive, $R^2 = 0.753$ (nOle e 1: 66.7% vs. *Olea*: 71.4%), Russian thistle, $R^2 = 0.782$ (nSal k 1: 66.1% vs. *S. kali*: 74.1%) and cypress, $R^2 = 0.757$ (nCup s 1: 25% vs. *Cupressus*: 23.2%). No correlation was found in the

others: grass (nPhl p 1: 29.5% and nPhp 5: 26.2% vs. grass pollen extract: 46.4%), London plane tree (rPla a 1 and nPla a 2: 6.3% vs. *Platanus*: 29.5%), mugwort (nArt v 1: 8.6% vs. *Artemisia*: 41.1%), pellitory (nPar j 1: 11.6% vs. *P. judaica*: 29.5%), birch tree (nBet v 1: 0% vs. *Betula*: 16.1%) or English plantain (nPla l 1: 8% vs. *P. lanceolata*: 43.8%).

Multivariate Analysis

Multivariate analysis (PP vs. PPR) showed that the factors having a causal relation with plant food sensitization were age ($p < 0.01$), sensitization to mugwort ($p < 0.01$) and sensitization to London plane tree ($p < 0.01$).

Sensitization to mugwort extract (1.418) and London plane tree extract (1.400) was directly proportional to the risk of suffering food allergy whereas the association with age (-0.56) was inversely proportional. The ORs for the variables age, mugwort and London plane tree were 0.95 (0.9–0.99), 4.2 (1.6–10.8) and 4.1 (1.4–11.5), respectively.

None of the confounding factors studied (mite allergy, latex allergy and dander allergy) were significant in the multivariate analysis.

Discussion

The relation between pollen and food has been described in numerous studies though few have examined a large number of plant-derived foods systematically [1–3, 12, 20]. In our study, the number of pollinoses with plant food sensitization (39.9%) was high compared with other studies [7, 22, 23], especially if we consider that our study did not include children, who more often suffer from food allergies. However, it should be noted that comparison between our findings and other studies is difficult as there exists great heterogeneity in the studies undertaken to date [24]. However, the last EuroPrevall study, using ImmunoCAP, showed that the relative prevalence of sensitization to different foods is more frequent than would be expected by chance [25].

Almost one third (30.9%) of the patients (group PPRb) showed clinical allergy to at least one of the plant foods studied. In a similar study on patients from different parts of Spain, the authors also found a high proportion of pollinosis in patients allergic to plant foods (66.5%) [20]. Unfortunately, although the methodology was very similar, we cannot compare these data since in that study, patient selection was made based on whether they were allergic to plant foods or not, independent of the pollinosis. In our study, we included patients sensitized to food but with no apparent clinical symptoms (PPRa group) as patients at risk of experiencing plant food allergy. We are aware of the discrepancies in the various reports concerning the classification of these patients, but even though this group was small in our sample (9.1%), the similarity of the clinical characteristics led us to classify them as patients at risk of plant food allergy (PPR) [20].

The pollinosis patients with and without food allergy shared similar demographic and comorbidity characteristics though the patients with pollinosis and food allergy were younger (28.2 years) than those without food allergy

Table 3. Sensitizations and clinical symptoms to plant food

Food	Sensitization, n	Clinical symptoms, n
Peach	63 (27.0)	48 (20.6)
Hazelnut	56 (24.0)	32 (13.7)
Mustard	54 (23.2)	17 (7.3)
Sunflower seeds	53 (22.7)	28 (12.0)
Peanut	52 (22.3)	28 (12.0)
Walnut	52 (22.3)	34 (14.6)
Almond	47 (20.2)	21 (9.0)
Lentil	44 (18.9)	2 (0.9)
Kiwi	31 (13.3)	12 (5.2)
Apple	30 (12.9)	15 (6.4)
Chestnut	23 (9.9)	10 (4.3)
Banana	22 (9.4)	10 (4.3)
Tomato	15 (6.4)	9 (3.9)
Soy	12 (5.2)	1 (0.4)
Melon	10 (4.3)	8 (3.4)

Figures in parentheses are percentages.

The table shows the distribution of sensitizations based on SPT and clinical symptoms taken from the self-reported questionnaire. The foods causing most problems in the patients in our area were peaches and nuts.

Table 4. Determinations of recombinant and purified allergens

Allergen	Sample	PP group	PPR group
Ole e 1	74 (66.7)	31 (70.5)	43 (64.2)
Ole e 9	3 (2.7)	1 (2.3)	2 (2.9)
Art v 1	20 (8.6)	6 (13.6)	14 (20.6)
Bet v 1	0	0	0
Cup s 1	28 (25.0)	11 (25.0)	17 (25.0)
Cyn d 1	27 (24.1)	9 (20.5)	18 (26.5)
Len c 1	4 (3.6)	0 (0.0)	4 (5.9)
Par j 1	13 (11.6)	5 (11.4)	8 (11.8)
Peroxidase	9 (8.0)	1 (2.3)	8 (11.8)
Phl p 1	33 (29.5)	12 (27.3)	21 (30.9)
Phl p 5	12 (10.8)	6 (13.6)	6 (9.0)
Pla a 1+2	7 (6.3)	3 (6.8)	4 (5.9)
Pla l 1	9 (8)	2 (4.5)	7 (10.3)
Chitinase	4 (3.6)	2 (4.5)	2 (2.9)
Che a 1	11 (9.8)	2 (4.5)	9 (13.2)
Che a 3	20 (17.9)	5 (11.4)	15 (22.1)
Profilin	6 (5.4)	1 (2.3)	5 (7.4)
Pru p 3	49 (43.8)	1 (2.3)	48 (70.6)
Sal k 1	74 (66.1)	31 (70.5)	43 (63.2)
Sin a 1	4 (3.6)	1 (2.3)	3 (4.5)

Figures in parentheses are percentages.

The table shows the distribution of positive results of recombinant and purified allergens for the whole sample and for each of the two study groups.

(40.6 years). This may be due to an increased prevalence of this disease as suggested by some authors, a change in the lifestyle of young persons, or other factors not detected by this study [26].

The distribution of pollen in our Mediterranean area was similar to that reported in other studies in the same area [27, 28]. The main sensitizations were to *Olea* spp. and *Salsola* spp., though they did not appear to be related to plant food allergy. Nevertheless, the distribution of the main sensitizations to pollen, apart from *Olea* spp. and *Salsola* spp., varied significantly depending on the study group. In the PP group, after *Olea* spp. and *Salsola* spp., the most frequent sensitizations were to Gramineae (40.4%), *Plantago* spp. (30.5%) and *Artemisia* spp. (19.9%), and in the PPR group, to *Artemisia* spp. (52.7%), *Plantago* spp. (48.4%) and Gramineae (48.4%). Indeed, in the groups displaying sensitization or clinical symptoms related to food, those pollens not present in the air caused a higher sensitization.

The foods producing most clinical symptoms in these patients (peach and nuts) agree with the results of other studies in the Mediterranean area [29]. On the other hand, sensitization to legumes (lentil 18.9% and soy 5.2%) caused few clinical symptoms (lentil 0.9% and soy 0.4%) and may have an important cross-reactivity component [30].

Molecular characterization showed that the allergen profile was similar to that found by skin testing. Determination of Pru p 3 may be useful in patients suspected of being allergic to plant foods (OR 3.3, 95% CI 2.3–4.8). Pru p 3 seems not only to act as a peach allergen but also as a marker of LTP, responding to sensitizations from the Rosacea family apart from peach, which shares the same epitopes. Besides, Pru p 3 might show cross-reactivity with LTP of botanically unrelated plant-derived foods [31]. In addition, as found in other studies, it may also be a marker of symptom severity [32]. Determination of Bet v 1, which has been much used in northern Europe because of the relation between birch pollinosis and apple allergy, did not prove so useful in our area [33]. Nor was Ole e 9 a good indicator of the risk of food allergy in our area, in agreement with another study [34]. The introduction of allergen microarrays may permit better optimization in the characterization of these patients [35].

Of interest was the fact that a significant correlation was only found between the extracts used and the main allergens in three cases (olive, Russian thistle and cypress). In these cases, other minority allergens related with the oral allergy syndrome (Ole e 2 and Sal k 4) are not as prevalent as in other studies [36], probably because

we do not have such high concentrations of olive pollen as in other areas of Spain, such as Jaen or Seville. Immunotherapy to treat pollinosis based on skin test diagnosis would seem justified in these cases, but with other pollens, further studies should be performed before starting immunotherapy.

Multivariate analysis showed that only the variables age and sensitization to the extracts of mugwort and London plane tree were important factors for classifying the patients with a risk of food allergy in our area. In fact, even though mugwort and London plane tree pollens are not present in the air, it would be very useful to incorporate them into the skin test battery to identify patients at risk of plant food allergy. Also, the discrepancies between positive skin tests with pollen extracts and negative IgE to recombinant markers of primary sensitization are suggestive of sensitization to pollen pan-allergens if the markers for primary sensitization are of food origin.

The relation between mugwort and London plane tree pollens and plant food is known. Associations have been described between mugwort and specific foods (spices, mustard and peach), and a higher frequency of the oral allergy syndrome has been reported in patients sensitized to London plane tree [31, 37, 38]. In our study, no association was found with any particular food or any specific allergen (Art v 1 or Pla a 1 and Pla a 2), but rather these extracts appeared to act as an LTP marker (Art v 3 and Pla a 3) [39].

Lauer et al. [40] have shown that the 10-kDa IgE-reactive protein purified from plane pollen is an nsLTP. This allergen, Pla a 3, was characterized as a minor allergen (27.3%) in plane pollen-allergic patients without food allergy and as a major allergen in plane pollen-allergic patients with peach allergy, showing a prevalence of IgE reactivity of 63.8% [40].

In conclusion, the results of this study show that plant food allergy is much more frequent in pollinosis. Accordingly, the risk of food allergy should be studied in all pollinosis patients. The introduction of extracts of mugwort (*A. vulgaris*) and London plane tree (*P. acerifolia*) as LTP markers in the battery of skin tests may help identify these patients in our Mediterranean area. Positive results should be studied with a wider panel of skin tests. And, finally, determination of Pru p 3 may be convenient in patients suspected of having plant food allergy as it would help confirm the allergy, and high levels would indicate the possible presence of more than one food allergy.

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Disclosure Statement

None of the authors have any conflict of interest.

References

- 1 Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC: IgE to food allergens are highly prevalent in patients allergic to pollen, with and without symptoms of food allergy. *Clin Exp Allergy* 1994;24:367–374.
- 2 Ghunaim N, Grönlund H, Kronqvist M, et al: Antibody profiles and self-reported symptoms to pollen-related food allergens in grass pollen-allergic patients from northern Europe. *Allergy* 2005;60:185–191.
- 3 Eriksson NE, Formgren H, Svenonius E: Food hypersensitivity in patients with pollen allergy. *Allergy* 1982;37:437–443.
- 4 Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization: allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108:147–334.
- 5 Fernandez Rivas M: Food allergy in Alergológica-2005. *J Investig Allergol Clin Immunol* 2009;19(suppl 2):37–44.
- 6 Ostblom E, Egmar AC, Gardulf A, Lilja G, Wickman M: The impact of food hypersensitivity reported in 9-year-old children by their parents on health-related quality of life. *Allergy* 2008;63:211–218.
- 7 Flabbee J, Petit N, Jay, et al: The economic costs of severe anaphylaxis in France: an inquiry carried out by the Allergy Vigilance Network. *Allergy* 2008;63:360–365.
- 8 de Blok BM, Vlieg-Boerstra BJ, Oude Elberink JN, et al: A framework for measuring the social impact of food allergy across Europe: a EuroPrevall state of the art paper. *Allergy* 2007;62:733–737.
- 9 Karisola P, Kotovuori A, Poikonen S, et al: Isolated hevein-like domains, but not 31-kD endochitinases, are responsible for IgE-mediated in vitro and in vivo reactions in latex-fruit syndrome. *J Allergy Clin Immunol* 2005;115:598–605.
- 10 Midoro-Horiuti T, Brooks EG, Goldblum RM: Pathogenesis-related proteins of plants as allergens. *Ann Allergy Asthma Immunol* 2001;87:261–271.
- 11 Bublin M, Pfister M, Radauer C, et al: Component-resolved diagnosis of kiwifruit allergy with purified natural and recombinant kiwifruit allergens. *J Allergy Clin Immunol* 2010;8.
- 12 Fernandez-Rivas M, Bolhar S, González-Mancebo E, et al: Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006;118:481–488.
- 13 Aleman A, Sastre J, Quirce S, et al: Allergy to kiwi: a double-blind, placebo-controlled, food challenge study in patients from a birch-free area. *J Allergy Clinical Immunol* 2004;113:543–550.
- 14 Dreborg S, Sub-Committee on Skin Test of the European Academy of Allergology and Clinical Immunology. Methods for skin testing. *Allergy* 1989;44(suppl 10):22–30.
- 15 Dreborg S, Sub-Committee on Skin Test of the European Academy of Allergology and Clinical Immunology. 6. Skin tests used for epidemiological studies. *Allergy* 1989;44:52–59.
- 16 EAACI: Skin tests used in type I allergy testing. Position paper. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989;44:1–59.
- 17 Ortolani C, Spano M, Pastorello EA, Ansaloni R, Magri GC: Comparison of results of skin pricks test (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989;83:683–690.
- 18 Ibañez MD, Alonso E, Blasco C, et al: Comité de Reacciones Adversas a Alimentos de la Sociedad Española de Alergología e Inmunología Clínica. Metodología diagnóstica en alergia a alimentos. *Allergol Inmunol Clin* 1999;14:50–62.
- 19 Petersen AB, Gudmann P, Milvang-Grønager P, et al: Performance evaluation of a specific IgE assay developed for the ADVIA Centaur immunoassay system. *Clin Biochem* 2004;37:882–892.
- 20 Cuesta-Herranz J, Barber D, Blanco C, et al: Differences among pollen-allergic patients with and without plant food allergy. *Int Arch Allergy Immunol* 2010;153:182–192.
- 21 Sociedad Española de Alergología e Inmunología clínica (Internet). Madrid (Reference made on 10th October, 2007). Available on <http://www.polenes.com/>.
- 22 Alvarado MI, Pérez M: Study of food allergy in the Spanish population. *Allergol Immunopathol (Madr)* 2006;34:185–193.
- 23 Rona RJ, Keil T, Summers C, et al: The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120:638–646.
- 24 Zuidmeer L, Goldhahn K, Rona RJ, et al: The prevalence of plant food allergies: a systematic review. *J Allergy Clin Immunol* 2008;121:1210–1218.
- 25 Burney P, Summers C, Chinn S, Hooper R, van Ree R, Lidholm J: Prevalence and distribution of sensitization to foods in the European Community Respiratory Health Survey: a EuroPrevall analysis. *Allergy* 2010;65:1182–1188.
- 26 Cruz NV, Wilson BG, Fiocchi A, Bahna SL, American College of Allergy, Asthma and Immunology Adverse Reactions to Food Committee: Survey of physicians' approach to food allergy. I. Prevalence and manifestations. *Ann Allergy Asthma Immunol* 2007;99:325–333.
- 27 Subiza J, Feo Brito F, Pola J, Moral A, Fernández J, Jerez M: Polenés alérgicos y polinosis en 12 ciudades españolas. *Rev Esp Allergol Inmunol Clin* 1998;13:45–58.
- 28 Fernández Sánchez J, García Más F, Esteban A, Miralles A: Incidencia de granos de polen y polinosis en la ciudad de Elche, 1995. *Rev Esp Allergol Inmunol Clin* 1998;13:88–91.
- 29 Carrasquer Moya C, Peláez Hernández A, Durá Calatayud M, Burchés Baixauli E, Bertó Salom JM, Sastre Sastre A: Allergy to peach. *Allergy* 2002;57:756–757.
- 30 Martínez San Ireneo M, Ibañez MD, Fernández-Caldas E, Carnés J: In vitro and in vivo cross-reactivity studies of legume allergy in a Mediterranean population. *Int Arch Allergy Immunol* 2008;147:222–230.
- 31 Asero R, Mistrello G, Roncarolo D, et al: Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy* 2002;57:900–906.
- 32 Rossi RE, Monasterolo G, Canonica GW, Passalacqua G: Systemic reactions to peach are associated with high levels of specific IgE to Pru p 3. *Allergy* 2009;64:1795–1796.

- 33 Bohle B: The impact of pollen-related food allergens on pollen allergy. *Allergy* 2007;62: 3–10.
- 34 Palomares O, Villalba M, Quiralte J, Polo F, Rodríguez R: 1,3-Beta-glucanases as candidates in latex-pollen-vegetable food cross-reactivity. *Clin Exp Allergy* 2005;35:345–351.
- 35 Jahn-Schimd B, Harwanegg C, Hiller R, et al: Allergen microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin Exp Allergy* 2003;33:1443–1449.
- 36 Scala E, Alessandri C, Bernardi ML, et al: Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010;40:911–921.
- 37 Enrique E, Cisteró-Bahíma A, Bartolomé B, et al: *Platanus acerifolia* pollinosis and food allergy. *Allergy* 2002;57:351–356.
- 38 Pastorello EA, Pravettoni V, Farioli L, et al: Hypersensitivity to mugwort (*Artemisia vulgaris*) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. *J Allergy Clin Immunol* 2002;110:310–317.
- 39 Lombardero M, Garcia-Selles FJ, Polo F, et al: Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy* 2004;34:1415–1421.
- 40 Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, Cistero-Bahíma A, Vieths S, Scheurer S: Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. *Clin Exp Allergy* 2007;37:261–269.