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Differences among Pollen-Allergic Patients with and without Plant Food Allergy

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Key Words
Pollen allergy · Plant food allergy · Component-resolved diagnostics · Pru p 3 · Profilin · Asthma · Immunotherapy

Abstract
Background: A considerable number of pollen-allergic patients develops allergy to plant foods, which has been attributed to cross-reactivity between food and pollen allergens. The aim of this study was to analyze the differences among pollen-allergic patients with and without plant food allergy. ***Methods:*** Eight hundred and six patients were recruited from 8 different hospitals. Each clinical research group included 100 patients (50 plant food-allergic patients and 50 pollen-allergic patients). Diagnosis of pollen allergy was based on typical case history of pollen allergy and positive skin prick tests. Diagnosis of plant-food allergy was based on clear history of plant-food allergy, skin prick tests and/or plant-food challenge tests. A panel of 28 purified allergens from pollens and/or plant foods was used to quantify specific IgE (ADVIA-Centaur® platform). ***Results:*** Six hundred and sixty eight patients (83%) of the 806 evaluated had pollen allergy: 396 patients with pollen allergy alone and 272 patients with associated food and pollen allergies. A comparison of both groups showed a statistically significant increase in the food and pollen allergy subgroup in frequency of: (1) asthma (47 vs. 59%; $p < 0.001$); (2) positive skin test results to several pollens: *Plantago, Platanus, Artemisia, Betula, Parietaria* and *Salsola* ($p < 0.001$); (3) sensitization to purified allergens: Pru p 3, profilin, Pla a 1 – Pla a 2, Sal k 1, PR-10 proteins and Len c 1. ***Conclusion:*** Results showed relevant and significant differences between both groups of pollen-allergic patients depending on whether or not they suffered from plant-derived food allergy.

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Introduction

Allergy is a common health problem in the general population. Recent epidemiological studies based on objective diagnostic methods indicate that pollen allergy is one of the most common chronic allergic disorders in modern society [1, 2]. Patients suffering from pollinosis frequently display adverse reactions after ingesting a wide range of plant foods [3, 4] and several associations between the two allergies have been described in the literature [5, 6]. IgE directed against common cross-reactive structures shared by pollen and plant food has been the most widely accepted and experimentally supported explanation for pollen-food syndrome [5, 7, 8]. Diagnosis using allergen extracts can serve to identify sensitization to a certain allergen source, but give no information regarding the molecular identity of the disease-eliciting allergen.

Advances in proteomic and molecular biology have allowed for both the identification and isolation as well as the cloning and the recombinant production of allergenic proteins to be used for diagnostic and therapeutic purposes. The ability to produce panels of purified allergens (natural or recombinant), defined as component-resolved diagnostics (CRD) [9], makes it possible to determine the frequency of IgE recognition of the individual allergens and has led to the quantification of specific IgE antibodies directed against a wide number of purified allergens from different allergenic sources [10]. Molecular analysis of the allergen sensitization pattern may serve to enhance the predictive and prognostic power of IgE antibody-based allergy diagnostics, as although certain allergens are known to be closely linked to manifestations of allergic disease, others, such as typically cross-reactive carbohydrate determinants, are considered to be only weakly associated with clinical reactivity [11, 12]. Thus, CRD make it possible to establish significant associations between particular subpopulations of specific IgE, measured by the use of individual allergen components, and clinically relevant aspects of the allergic disease. However, limited information is available concerning this matter, and evaluation of a large number of patients classified according to well-defined groups and using panels of many allergens will help to improve and optimize the management of allergic patients and lead to a better knowledge of the real meaning of positive results to each molecule. Stekelbroeck et al. [13] recently attracted attention to this matter by performing well-designed high-power studies to substantiate current and future findings.

A considerable percentage of pollen-allergic patients develops allergy to plant foods [4, 14], and to date no study has compared differences among pollen allergic patients depending on whether or not they have plant food allergy. Such an analysis would prove highly useful for clinicians in their effort to optimize diagnosis and treatment of pollen-allergic patients. Therefore, this study aims to analyze the differences between these two groups of pollen-allergic patients, both with and without plant food allergy.

Methods

Study Design

The study was carried out within the context of the Vegetalia Network, a cooperative research effort devoted to the investigation of plant-derived food allergy and supported by the Spanish Ministry of Health and Consumer Affairs. The study consisted of several stages. Firstly, patients were recruited from each participating hospital and evaluated for pollen and/or plant food allergies. Then, a serum sample was obtained from each patient and stored frozen. In addition, clinical data on the patients with the pollen and/or plant food allergy were collected by the clinicians and included in a database that had been specially designed by the Vegetalia network. Meanwhile, non-clinician researchers prepared a panel of natural or recombinant purified allergens involved in pollen or plant-derived food allergy. Afterwards, specific IgE to these purified allergens was quantified in the patients' sera by using the ADVIA-Centaur® platform (Siemens Med. Sol. Diagnostics Europe Ltd., Saint Vulbas, France) [10]. These results were also included in the database. Finally, a statistical evaluation of results included in the database was performed.

The study protocol was approved by an ethical committee from each participating hospital and patients gave their written consent to take part in the study.

Geographical Area of the Study

Participating in this study were 11 research groups from different areas of Spain – Las Palmas de Gran Canaria (Canary Islands), Jaén (Andalusia), Pamplona (Navarre), Barcelona (Catalonia) and Madrid (Madrid) – all with significant differences among them in terms of airborne pollens and their concentrations (www.polenes.com). This diversity can be easily observed by looking at the pollen calendar (fig. 1). In the area of Jaén, land is used for intensive olive cultivation, reporting maximum *Olea* pollen peaks (for example 15,500 grains/m^3 during the 2003 pollen season). Madrid, in the central area of Spain, consists of a plateau (average of 600 m above sea level) characterized by a continental climate. *Gramineae* pollens are the most important pollens eliciting allergy in the Madrid area. *Gramineae* pollens are also important in the environment of Pamplona, although in general *gramineae* pollen counts are lower. Barcelona has a Mediterranean climate, where *Parietaria* pollens elicit the highest counts of this pollen among the five cities involved in the study. The Canary Islands exhibit the lowest pollen concentrations of the 5 regions due to the high humidity. Nonetheless, *Artemisia* pollens are important allergens but mites are the most common allergens in this area.

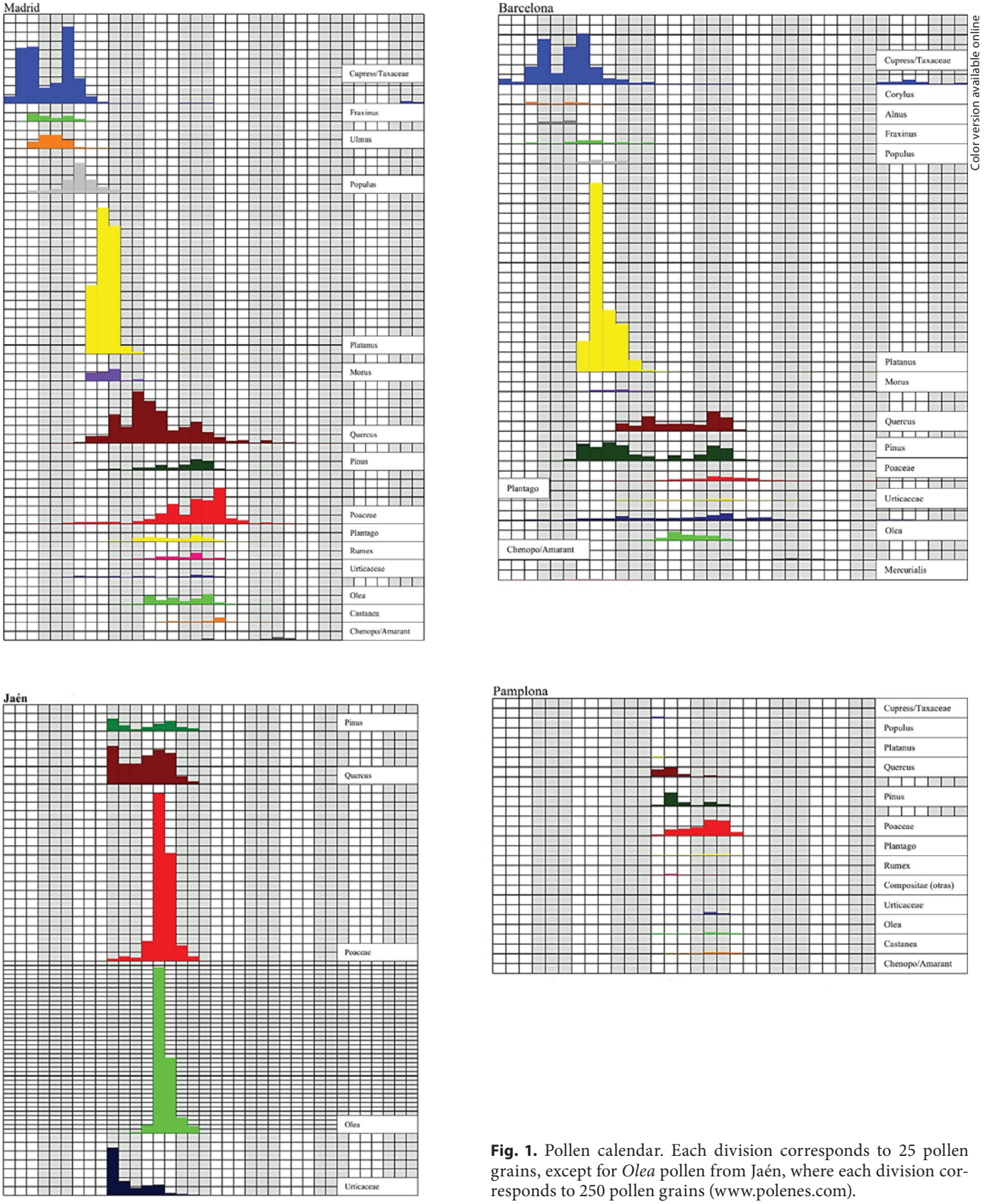

Fig. 1. Pollen calendar. Each division corresponds to 25 pollen grains, except for *Olea* pollen from Jaén, where each division corresponds to 250 pollen grains (www.polenes.com).

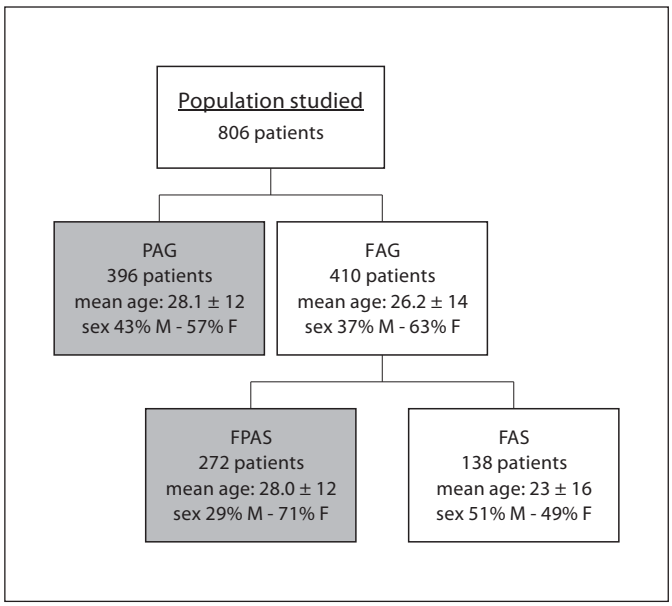

Fig. 2. Characteristics of patient groups. M = Male; F = female.

Patients

Patients were recruited by 8 clinical groups located in 5 different cities across Spain. Each clinical group recruited 100 patients divided into 2 different groups of 50 patients each: the pollen-allergy group (PAG) and the plant food-allergy group (FAG).

Criteria for inclusion in the PAG were consecutive patients with a compatible clinical history of pollinosis confirmed by positive skin tests to pollen extracts; patients with plant food allergy were excluded. Patients with positive skin tests to any plant food but displaying no symptoms on eating the food were not excluded from this group (latent atopy). Allergic rhinitis to pollen was defined as recurrence of typical symptoms of rhinitis (that is, repeated sneezing, nasal itching, watery rhinorrhea and nasal congestion) and allergic asthma to pollen when patients experienced recurrent episodes of wheezing, breathlessness, chest tightness and coughing during each pollen season, confirmed by positive skin test results to pollen extracts.

Criteria for inclusion in the FAG were consecutive patients diagnosed with plant-food allergy. Plant-food allergy was diagnosed in patients having a clear history of plant-food allergy adverse reactions, suggestive of IgE-mediated allergy, showing positive skin prick tests and/or food challenge tests, following the diagnostic algorithm of the Food Adverse Reaction Committee of Sociedad Española de Alergia e Inmunología Clínica (http://revista.seaic.es/april99/50-62.pdf) [15]. Patients suffering severe systemic reactions to plant foods, as well as patients with typical, recent, repeated and unequivocal reactions who had positive skin tests did not undergo an oral challenge test to diagnose plant-food allergy (http://revista.seaic.es/april99/50-62.pdf) [15].

Once patients were studied and included in the database, the FAG was divided into 2 subgroups (fig. 2): plant food -allergic patients who were allergic to pollen (FPAS) and plant food-allergic patients without pollen allergy (FAS).

Exclusion criteria were pregnancy or lactation period, extensive skin disease, serious psychiatric/psychological disturbances, contraindication to adrenaline treatment, alcohol or drug addiction, treatment with β-blockers, as well as any other condition which would either hamper protocol compliance or for which an oral challenge test is contraindicated [16].

A serum sample from each patient was taken during the first visit and was kept frozen at –80°C until used.

Skin Prick Tests

Skin prick tests were performed with a commercial battery (ALK-Abelló, Madrid, Spain) of pollen extracts, including *Lollium perenne, Betula verrucosa, Cupressus sempervirens, Platanus acerifolia, Artemisia vulgaris, Parietaria judaica, Salsola kali, Plantago lanceolata* and *Olea europaea,* as well as plant-food extracts (peach, mustard, almond, hazelnut, peanut, chestnut, sunflower seed, lentil and soy) and latex extract. The ALK-Lancet needle (ALK-Lancet; ALK-Abelló, Horsholm, Denmark) was used for skin tests, which were performed according to EAACI guidelines [17]. Skin tests to fresh plant foods (apple, muskmelon, banana, kiwi and tomato) were performed by prick-prick testing, following the technique described by Dreborg and Foucard [18]. Prick-prick testing to other plant foods was also performed when patients suffered reactions to plant foods non included in the battery or when commercial extracts elicited a negative result. Histamine phosphate at 10 mg/ml and normal saline solution were used as positive and negative controls, respectively. A wheal with a diameter at least 3 mm larger than the negative control was considered a positive reaction.

Panel of Purified Allergens

The following natural or recombinant allergens, isolated by previously described methods, were included in the diagnostic panel: *Phleum pratense:* nPhl p 1 [19] and nPhl p 5 [20]; *Cynodon dactilon:* nCyn d 1 [21]; *A. vulgaris:* nArt v 1 [22]; *O. europaea:* nOle e 1 [23] and Ole e 9, as a mix of rN-terminal domain and rC-terminal domain from Ole e 9 [24, 25]; *P. lanceolata:* nPla l 1 [26]; *P. judaica:* nPar j 1 [27]; *C. sempervirens:* nCup s 1 [10]; *S. kali:* nSal k 1 [28]; *Chenopodium album:* rChe a 1 [29], rChe a 2 (profilin) [30] and rChe a 3 (polcalcin) [31]; *P. acerifolia*: rPla a 1 and nPla a 2 (BIAL-Aristegui, Bilbao, Spain). As pan-allergens, we used polcalcin: rChe a 3 from *C. album* pollen [31]; nonspecific LTP from peach: rPru p 3 [32]; a mixture of 3 profilins: rCuc m 2 from muskmelon [33], rChe a 2 from *C. album* pollen [30] and rMal d 4 from apple [10]. We also tested purified proteins from wheat flour: nCM3 and nCM16 (α-amylase inhibitor subunits) [34]; lentil: nLen c 1 (vicilin) [35]; a mixture of 2 chitinases: n Pers a 1 from avocado [36] and rCas s 5 from chestnut [36]; a mixture of PR-10 protein: nBet v1 from *B. verrucosa* [37] and rMal d 1 from apple [38]; nSin a 1, a 2S albumin from mustard [39]; peroxidase as a CCD marker (Sigma-Aldrich Co. St. Louis, Mo., USA).

Ole e 1, Ole e 9, Che a 1, Che a 2, Che a 3, Sal k 1 and Sin a 1 were supplied by Facultad de Químicas, Universidad Complutense (Madrid, Spain); Pru p 3, Cuc m 2, CM3, CM16, Len c 1, Pers a 1 and Cas s 5 by ETS Ingenieros Agrónomos (Madrid, Spain); and Phl p1, Phl p 5, Cyn d 1, Art v 1, Pla l 1, Par j 1, Cup s 1, Bet v 1, Mal d 1 and Mal d 4 by ALK-Abelló (Madrid, Spain). Pla a 1 and Pla a 2 were purchased from BIAL-Aristegui.

Table 1. Frequency of positive skin test results to pollen extracts in both pollen patient groups in several centres

	Group	*Gramineae*	*Olea*	*Cupressus*	*Platanus*	*Plantago*	*Artemisia*	*Betula*	*Parietaria*	*Salsola*
Jaén	PAG	66%	89%	26%	20%	34%	23%	20%	25%	23%
	FPAS	73%	90%	29%	57%	69%	57%	57%	37%	61%
Las Palmas	PAG	71%	42%	17%	17%	27%	56%	10%	19%	10%
	FPAS	43%	37%	21%	49%	43%	71%	29%	19%	23%
Barcelona	PAG	63%	34%	47%	45%	35%	39%	29%	35%	10%
	FPAS	50%	33%	42%	58%	34%	29%	34%	26%	24%
Pamplona	PAG	88%	36%	21%	12%	19%	10%	4%	6%	12%
	FPAS	90%	60%	43%	57%	60%	33%	37%	23%	10%
Madrid										
12 Octubre	PAG	82%	66%	59%	57%	59%	51%	35%	29%	39%
	FPAS	79%	67%	49%	72%	69%	67%	49%	44%	69%
Niño Jesús	PAG	86%	68%	48%	34%	14%	16%	24%	14%	10%
	FPAS	89%	63%	56%	58%	42%	37%	47%	32%	47%
FJD	PAG	81%	64%	49%	45%	40%	32%	13%	9%	15%
	FPAS	94%	77%	64%	88%	88%	70%	58%	39%	58%
F. Alcorcón	PAG	94%	74%	44%	42%	48%	40%	24%	14%	20%
	FPAS	100%	89%	55%	86%	79%	62%	55%	3%	76%

12 Octubre = Hospital 12 Octubre (Madrid); Niño Jesús = Hospital del Niño Jesús (Madrid); FJD = Fundación Jiménez Díaz (Madrid); F. Alcorcón = Fundación Alcorcón (Alcorcón).

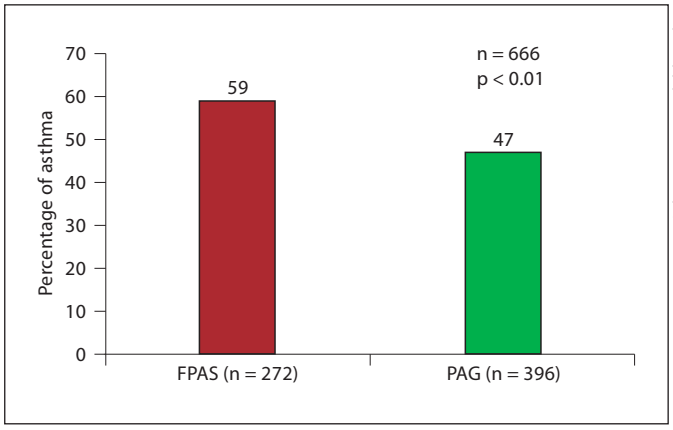

Fig. 3. Differences in frequency of asthma between pollen-allergic patients with and without plant-food allergy.

Color version available online

Specific IgE Determination

The level of specific IgE to the different allergens in patient sera was tested on the ADVIA-Centaur platform. The principle of the specific IgE assay was based on a reverse sandwich assay and performed as previously described [10, 40].

Statistics

Statistical analysis was performed with SPSS (SPSS Inc., Chicago, Ill., USA). Descriptive statistics included frequency of positive results with 95% CI for qualitative variables. For quantitative variables, means and SD were calculated, and for specific IgE and SPT results, medians and 25th (Q1) and 75th (Q3) percentiles were given. A χ^2 test was used for comparisons of frequencies. Values were considered significant at a p value of less than 0.05.

Results

Description of Patients

Eight hundred and six patients – 321 male (39.8%) and 485 female (60.2%) – were evaluated. On analyzing patients included in the study, 396 patients (49.1%) belonged to the PAG and 410 patients (50.9%) to the FAG. A subgroup of 149 patients of the 410 plant food-allergic patients (41%) needed an oral challenge test to confirm the diagnosis of food allergy. The FAG was divided in 2 subgroups: 272 patients (66.5%) with food and pollen allergy (FPAS) and 138 patients (33.5%) with food allergy but without pollen allergy (FAS) (fig. 2). In this study we reported results from the 668 pollen-allergic patients: 396 patients without food allergy (PAG) and 272 patients allergic to plant foods and pollens (FPAS), excluding food-allergic patients without pollen allergy (FAS) (fig. 2).

Age and sex are shown in figure 2, showing a statistical predominance of female patients (PAG: 43% male and 57% female vs. FPAS: 29% male and 71% female; $p < 0.05$).

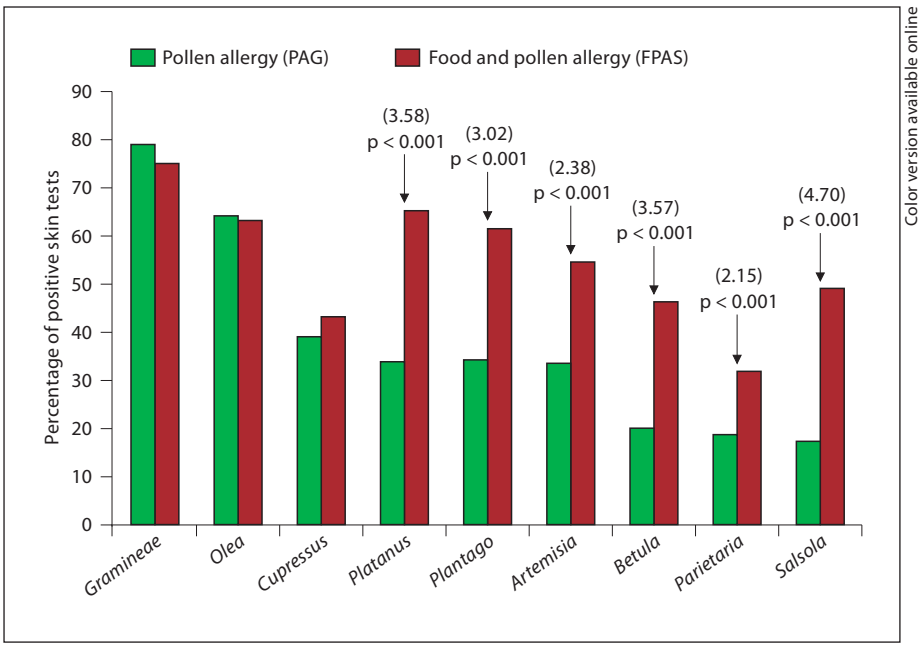

Fig. 4. Differences in frequency of positive skin prick tests between pollen-allergic patients with and without plant-food allergy. Odds ratios are given in parentheses.

Regarding symptoms of pollen-allergic patients as a whole (PAG and FPAS), 48% had rhinitis and 52% rhinitis and asthma. A statistically significant increase of asthma frequency was found when pollen-allergic patients also had allergy to any plant-derived food (47% PAG vs. 59% FPAS, $p < 0.001$) (fig. 3).

The 5 most frequent plant foods eliciting allergy among plant food-allergic patients with pollen allergy (FPAS) were: peach (48.9%), muskmelon (36.0%), kiwi (32.4%), chestnut (27.6%) and apple (19.1%). Other plant foods elicited allergic reactions with less frequency.

Skin Prick Tests

Frequencies of positive skin prick test responses to pollen extracts in both patient groups (with and without plant food allergy) are shown in figure 4. An analysis of skin test results to pollen extracts in the PAG shows that *Gramineae* pollens were found to elicit positive results in 79% of patients, *Olea* pollen in 64%, followed by *Cupressus* in 39%. *Plantago, Platanus* and *Artemisia* pollens elicited positive responses in 33–34% of the patients. Finally, *Betula, Parietaria and Salsola* elicited positive responses in less than 20% of patients in the PAG. As it is shown in table 1, there were important differences in pollen skin test results among different areas of the study, reflecting differences in pollen exposure both in the kind of pollens and concentrations.

It is worth stressing that significant differences were found on comparing the previous data with those from the FPAS. Differences were relevant – roughly double – and highly statistically significant for: *P. lanceolata* 34 vs. 61%, $p < 0.001$; *P. acerifolia* 34 vs. 65%, $p < 0.001$; *A. vulgaris* 33 vs. 54%, $p < 0.001$; *B. verrucosa* 20 vs. 46%, $p < 0.001$; *P. judaica* 18 vs. 32%, $p < 0.001$; *S. kali* 17 vs. 49%, $p < 0.001$. There were minor and statistically nonsignificant differences in the percentage of positive skin test results between both groups for *Gramineae, Olea* and *Cupressus* pollen extract. Table 1 shows skin test results in both patient groups in each area of the study.

Molecular Pattern of Sensitization

The frequencies of positive results of specific IgE to purified allergens are shown in figure 5. The most frequent molecules eliciting sensitization in the PAG were the major grass pollen allergens Phl p 1 (66.3%) and Cyn d 1 (46.4%), followed by Ole e 1, the major olive pollen allergen (46.2%). Other frequent sensitizing molecules were Phl p 5 (38.8%) and Cup s 1 (34.4%). These results correlate with the most frequent pollen extracts eliciting sensitization *(Gramineae, Olea* and *Cupressus)* attending to skin prick test results. Sensitization to all other molecules tested was lower than 13%. Moreover, we would like to highlight two interesting findings in this PAG group: 13% of patients were sensitized to profilin and 7.7% to lipid transfer protein (Pru p 3) in the PAG and these patients

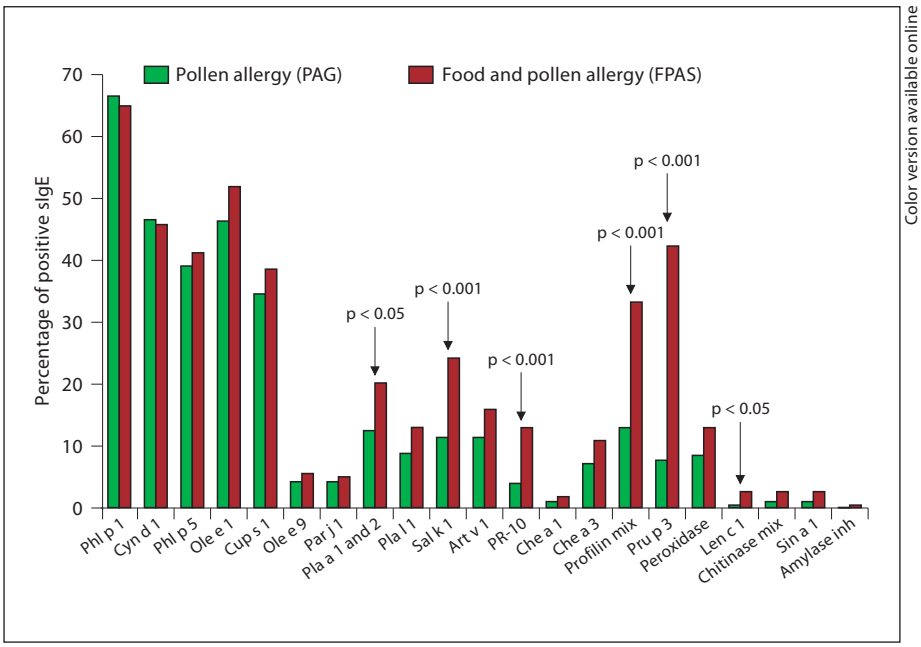

Fig. 5. Differences in frequency of positive specific IgE to purified allergens between pollen-allergic patients with and without plant-food allergy.

had never experienced any allergy symptoms related to plant-derived food (latent atopy).

Relevant and statistically significant differences were found between both subgroups of patients (PAG and FPAS) for major pollen allergens: Pla a 1 + Pla a 2 (12.5 vs. 20.3%; $p < 0.05$) and Sal k 1 (11.5 vs. 24.3%; $p < 0.001$); pollen and food allergens such as profilin (13 vs. 33.2%; $p < 0.001$) or PR-10 proteins (3.8 vs. 13.1%; $p < 0.001$); plant-derived food allergens such as Len c 1 (0.5 vs. 2.6%, $p < 0.05$) or Pru p 3 (7.7 vs. 42.2%; $p < 0.001$). Pru p 3 (42%) and profilin (33%) were the two allergens in FPAS whose differences in frequency of sensitization had the highest increase in relation to PAG (fig. 5), affecting up to 63% (59.2–66.7) of the patients in the FPAS. Differences in pollen exposure elicited varying frequencies of sensitization to purified proteins, as can be clearly observed in table 2. Frequency of sensitization to Ole e 9 is a particularly illustrative example of this fact, since sensitization to Ole e 9 was only relevant in areas exposed to very high concentrations of olive pollen grains, such as in Jaén.

Discussion

This study was carried out in the context of a research network on food allergy (Vegetalia Network) and performed as a multicentre study involving a large number of patients, thus reinforcing its results.

The diagnosis and treatment of pollen allergy have been based on anamnesis and results of skin test or specific IgE to pollen extracts. In this study we have analyzed 2 well-defined pollen-allergic patient groups and tested a wide and well-documented panel of purified allergens [10, 19–39]. Clinical data were compared with both the results obtained with the specific IgE responses to this panel and with a battery of commercially available pollen extracts used for skin tests. The results of this study showed significant and relevant differences between both groups of pollen-allergic patients depending on whether or not they suffered from plant-derived food allergy. This should be borne in mind in the management of pollen-allergic patients both for diagnosis and therapeutic decisions.

There was a higher prevalence of females among pollen-allergic patients in both patient groups (PAG and FPAS), but on comparing them this predominance of female patients was higher in the FPAS (71%) than in the PAG (57%), representing a statistically significant difference. Both of these facts support the findings reported in the review published by Jensen-Jarolim et al. [41], which states that the prevalence of allergy was greater in women from adolescence onwards, but also, that both asthma and food allergies were more frequently diagnosed in females.

Substantial differences were found in the frequencies of pollen eliciting sensitization across the different areas

Table 2. Frequency of specific IgE to purified proteins in different centres of the study

	Group	Phl p 1	Phl p 5	Cyn d 1	Ole e 1	Ole e 9	Cup s 1	Pla a 1+2	Pla l 1	PR-10	Art v 1	Par j 1	Che a 1	Che a 3	Sal k 1	Pro-filin	Pru p 3	Perox	Len c 1	Chi-tin	Sin a 1
Jaén	PAG	60%	32%	35%	82%	35%	40%	17%	10%	2%	7%	2%	2%	10%	27%	10%	5%	15%	0%	0%	3%
	FPAS	58%	29%	33%	77%	27%	42%	29%	28%	17%	7%	4%	8%	27%	42%	37%	44%	19%	2%	4%	7%
Las Palmas	PAG	34%	12%	30%	24%	0%	6%	4%	10%	6%	30%	2%	2%	4%	8%	4%	16%	10%	0%	2%	2%
	FPAS	31%	14%	23%	14%	0%	6%	9%	6%	14%	22%	0%	0%	3%	14%	14%	51%	9%	0%	3%	3%
Barcelona	PAG	44%	26%	34%	24%	0%	36%	30%	4%	2%	10%	22%	0%	6%	6%	10%	4%	4%	0%	0%	1%
	FPAS	38%	16%	20%	24%	0%	19%	24%	3%	13%	8%	24%	0%	5%	11%	5%	43%	0%	0%	3%	3%
Pamplona	PAG	84%	64%	56%	31%	0%	16%	4%	5%	2%	4%	2%	0%	4%	13%	7%	9%	4%	0%	0%	0%
	FPAS	79%	48%	59%	48%	0%	28%	7%	10%	28%	11%	0%	0%	10%	7%	31%	52%	10%	3%	3%	10%
Madrid																					
12 Octubre	PAG	70%	31%	43%	40%	2%	53%	8%	8%	6%	19%	2%	2%	17%	13%	15%	8%	8%	0%	0%	0%
	FPAS	71%	55%	58%	60%	0%	50%	29%	18%	5%	16%	5%	0%	8%	42%	53%	18%	13%	5%	5%	0%
Niño Jesús	PAG	74%	36%	52%	66%	4%	34%	4%	14%	4%	3%	2%	2%	6%	10%	10%	10%	6%	4%	2%	0%
	FPAS	89%	52%	68%	68%	5%	56%	17%	16%	16%	11%	0%	0%	0%	32%	53%	53%	26%	10%	0%	5%
FJD	PAG	76%	40%	48%	54%	0%	44%	20%	10%	6%	4%	2%	0%	2%	4%	18%	8%	14%	0%	2%	2%
	FPAS	85%	60%	48%	57%	3%	51%	12%	15%	6%	5%	0%	3%	12%	9%	30%	48%	15%	3%	0%	6%
F. Alcorcón	PAG	86%	64%	70%	56%	0%	50%	14%	8%	2%	15%	0%	0%	10%	14%	30%	0%	8%	0%	0%	2%
	FPAS	90%	69%	79%	65%	0%	69%	28%	14%	7%	12%	0%	0%	10%	31%	52%	34%	17%	0%	0%	0%

12 Octubre = Hospital 12 Octubre (Madrid); Niño Jesús = Hospital del Niño Jesús (Madrid); FJD = Fundación Jiménez Díaz (Madrid); F. Alcorcón = Fundación Alcorcón (Alcorcón); Chin = chitinase; Perox = peroxidase.

included in the study (table 1), though when taking all patients in the PAG as a whole, *Gramineae* were the most frequent pollens eliciting sensitization, followed by *O. europaea* and *C. sempervirens* pollens. On comparing these results with those of the subgroup of pollen-allergic patients with plant food allergy (FPAS), notable and statistically significant increases in positive skin prick test frequencies to several weed and tree pollen extracts are herein evaluated. Such a marked increase diminishes the value of skin tests for diagnosing pollen allergy in patients suffering from plant-derived food allergy since, in many cases, they were sensitized to pollens not found in their environment.

An interesting result of this study was the finding that Ole e 9 was a good marker of exposure to high concentrations of *Olea* pollen, which has been previously reported [10].

When comparing both groups (PAG and FPAS) at the molecular level, a significant increase was found both in pollen proteins (Pla a 1/Pla a 2, Sal k 1), pollen and food proteins (PR-10, profilin), and food proteins (Len c 1, Pru p 3). These findings are relevant but as of yet unknown, in spite of the important clinical consequences they could have on decision making both in diagnosis and treatment. Polysensitization makes accurate evaluation of pollen-allergic patients difficult, and therefore anamnesis and sensitization testing to whole pollen extracts may not be sufficient for correct diagnosis. For this reason, it is necessary to perform the analysis with purified allergens (CRD) to optimize the management of these patients [9, 12, 13, 42]. In addition, these results suggest that when evaluating pollen-polysensitized patients, concomitant plant food allergy has to be accounted for.

Pru p 3 (42%) and profilin (33%) were the two allergens in FPAS whose differences in frequency of sensitization had the highest increase in relation to PAG (fig. 5), affecting up to 63% (59.2–66.7) of the patients in the FPAS. This high prevalence of Pru p 3 and profilin sensitization in FPAS was consistent with other reports from Spain [33, 43, 44]. Nonetheless, we should highlight the high frequency of sensitization to Pru p 3 in spite of the fact that all patients were pollen allergic and that Pru p 3 fulfils criteria to be classified as a class I food allergen.

A very relevant finding of this study was the third increased allergen recorded in the pollen and plant food group: Sal k 1, a pectin methyl esterase, not previously associated with plant food allergy. Pla a 1 and Pla a 2 are major allergens of *P. acerifolia* which some reports had cited as being involved in plant food allergy [45, 46]. Although *P. acerifolia* pollens have been frequently associated with plant food allergy (peach, nuts and others) due to Pla a 3 – an LTP with partial cross-reactivity to Pru p 3 [47] – our data support the notion that Pla a 1 and Pla a 2 are potentially involved in these cross-reactivity reac-

tions. Finally, although birch pollen *(Betula)* is not a prevalent pollen in Spain [48], increased sensitization to a mix of Bet v 1 and Mal d 1 (PR-10) was also found in patients who were allergic to plant foods. Future evaluations should be performed to elucidate if this increase in PR-10 is elicited via apple allergy (Mal d 1), birch allergy (Bet v 1) or allergy to trees taxonomically related to birch.

When analyzing the group of pollen-allergic patients without plant food allergy (PAG), it would be worthwhile to emphasize that positive sensitizations to profilin and Pru p 3 were also detected, though recognition frequencies in both cases were not especially high. To the best of our knowledge, both data are relevant and deserve to be highlighted given that: (1) on the one hand, while it has been reported that frequency of Pru p 3 was low among pollen-allergic patients [10] – a group in which plant food-allergic patients were not excluded [10] – this is the first time that the frequency of positive results to Pru p 3 in non-plant food-allergic patients is reported, and (2) on the other hand, the percentage of positive results to profilin among non-plant food-allergic patients was very low and, consequently, sensitization to profilin out of the context of plant food allergy is infrequent. This was confirmed by results from each hospital participating in the study (table 2), thus offering new insight into previous findings [49, 50]. We believe that both circumstances were likely attributable to latent atopy as described by Juhlin-Dannfeldt [51] possibly occurring in the early stages of plant food allergy, and these patients will probably develop food allergy symptoms in the near future. Additional studies should be carried out to verify this hypothesis by performing a follow-up of profilin- and Pru p 3-sensitized patients without symptoms to any plant food.

Finally, the higher frequency of positive skin tests to several pollens and also the increase in the prevalence of positive results to some molecules were accompanied by an increase (12%) in frequency of asthma, which was statistically significant ($p < 0.01$). The major frequency of asthma among pollen-allergic patients with plant food allergy has already been reported, not only in previous studies focusing on plant food allergies [52–55] but also on polysensitized patients [56], with both facts manifested jointly in most of our patients. Our data support their claim, confirming it in a high-powered study.

Vaccine administration for allergic diseases is a worthwhile therapeutic option for treating pollen-allergic patients, and CRD [9] allows for optimal management of polysensitized patients, thus enabling clinicians to know if polysensitization in a particular patient is the result of true sensitization to several pollens or, conversely, the result of cross-reactivity to panallergens such as profilin and polcalcin. The results of our study clearly prove that associated plant food allergy influences the profile of pollen-allergic patients, and requires different diagnostic and treatment decisions, thereby making CRD an important tool to be used in the evaluation of these pollen-allergic patients.

In summary, pollen-allergic patients with an associated plant food allergy make up a special subgroup of pollen-allergic patients and deserve special attention in management due to the increased severity of the disease (that is, higher frequency of asthma), higher frequency of polysensitization and a different pattern of specific IgE to several purified allergens.

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