

Original article

Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study

Background: Allergy diagnosis in patients exposed to multiple pollen species is complex and misdiagnosis is often a cause for unsuccessful specific immunotherapy.

Objective: We studied the sensitization profile of individual allergens (major, minor and pan-allergens) in pollen-sensitized patients in a region with high exposure to olive pollen by investigating the influence of minor allergens on allergic disease and the association between pan- and minor allergen sensitizations.

Methods: A panel of 13 purified allergens, which included the most relevant allergens in the area, as well as minor olive allergens and pan-allergens, were screened using a high-capacity technology (ADVIA-Centaur®) in 891 patients.

Results: Olive allergy as measured by specific IgE to Ole e 1 was the leading pollinosis in the area. The minor olive allergens Ole e 7 and Ole e 9 were markers of more severe allergic illness. Profilin sensitization was associated mainly with grass allergy, the second most prevalent pollinosis. *Salsola kali* pollen allergy was the third most common cause of pollinosis in the area. The prevalence of sensitization to the peach allergen Pru p 3, a nonspecific lipid-transfer protein, was notable.

Conclusion: Epidemiological analysis by component-resolved diagnosis is a new method, which elucidates the interaction between allergen exposure gradient and patient sensitization. High exposure leads to differential sensitization profiles some of which are associated with more severe allergic conditions. Profilin sensitization, related mainly to grass pollinosis, was a marker of more severe grass pollen sensitization.

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Allergens may be classified according to population-based IgE prevalence (major or minor allergens), their physicochemical structure or biological role in the organism. Major allergens are defined as those recognized by more than 50% of the patients allergic to a particular source (1). Normally, a given source of allergens contains one or two major allergens; minor allergens are recognized in fewer than 50% of the patients. Pan-allergens constitute families of homologous and structurally related proteins from different species (i.e. profilins, lipid-transfer proteins, polcalcins, etc.) (2–5) responsible for extensive IgE cross-reactivity among a variety of allergenic sources. These characteristics hinder the identification of the

primary sensitizing agent in most patients sensitized to pan-allergens, making common diagnostic procedures based on crude extracts unspecific, which poses an obstacle to the development of specific allergic vaccines for immunotherapy.

The impact of pan-allergenicity is lower in patients living in geographical areas, such as central and northern Europe, where grass (mostly in central Europe) and Betulaceae (mostly northern Europe) have clearly distinct pollen seasons and are almost the only source of allergenic pollen. However, the situation is more problematic for patients living in geographical areas such as southern Europe, where grasses are still a relevant cause of pollinosis. In addition, other pollen species, such as olive, pellitory, cypress and Russian thistle, among others, play a significant role (6–8) as allergen sources particularly because their pollen seasons frequently occur

Abbreviations: CI, confidence interval; CV, coefficient of variation; LTP, nonspecific lipid-transfer protein; OR, odds ratio; sIgE, specific IgE.

in close succession or even overlap. Furthermore, in some parts of this geographical region, with peak counts above 10 000 grains/m³, minor allergens that rarely sensitize allergic patients in normally exposed areas become major allergens.

A case in point is olive pollen, a problem that has led to a different standardization strategy for allergy vaccines based on the quantification of relevant minor allergens (9, 10). Some patients may present more severe allergies, especially those sensitized to Ole e 10 have been reported to suffer from severe asthma (11). Patients sensitized to Ole e 7 [nonspecific lipid-transfer protein (LTP) of olive] or Ole e 9 (β -glucanase), but not to pollen pan-allergens polcalcin or profilin, were less tolerant of immunotherapy at the recommended allergen dose (12). A larger number of adverse reactions were recorded in patients sensitized to Ole e 7 or Ole e 9, but not sensitized to pan-allergens (12).

Moreover, Ole e 7 has been shown to be associated with an increased risk of food anaphylaxis (13). A previous study as part of an epidemiological survey of the pollen/food-allergic population (Red Vegetalia, unpubl. data; project number G3/094) found that in an area with an extremely high olive pollen exposure, the prevalence of the pan-allergens profilin and polcalcin was not statistically significantly higher than the prevalence in less exposed areas, indicating that pan-allergens might not be relevant olive allergens and that sensitization to these might be caused by other pollen species.

To verify and extend these preliminary results, we carried out an epidemiological study in 891 patients in collaboration with 34 clinical research and 3 basic research groups. A panel of specific molecular allergens comprising 13 purified allergens was developed and tested with a high-throughput technology (ADVIA-Centaur®, Bayer HealthCare Diagnostics Division, Tarrytown, NY, USA). The panel included major allergens of the relevant pollen species in the area, two minor olive allergens and three pan-allergens. Our main objective was to study the prevalence of minor olive allergens in olive-exposed areas. Other aims were to determine the association between pollen pan-allergens and major allergens of specific pollen species and to propose practical conclusions that might enhance diagnostic accuracy and aid in the development of effective allergy vaccines.

Methods

Geographical area of study

The area covered by the study is shown in Fig. 1. In some of the southern areas, up to 40% of the surface is used for intensive olive cultivation. These areas report maximum olive pollen peaks (e.g. 13 500 grains/m³, Jaén, 2003 pollen season) (14). The southeastern region is semiarid with low grass pollen counts, whereas the western region, influenced by Atlantic winds, reports the highest grass pollen counts (e.g. up to 1920 grains/m³, Badajoz, 2006 pollen season) (14). The central area of Spain consists of a plateau (average 600 m above sea level) characterized by a continental climate. Therefore,

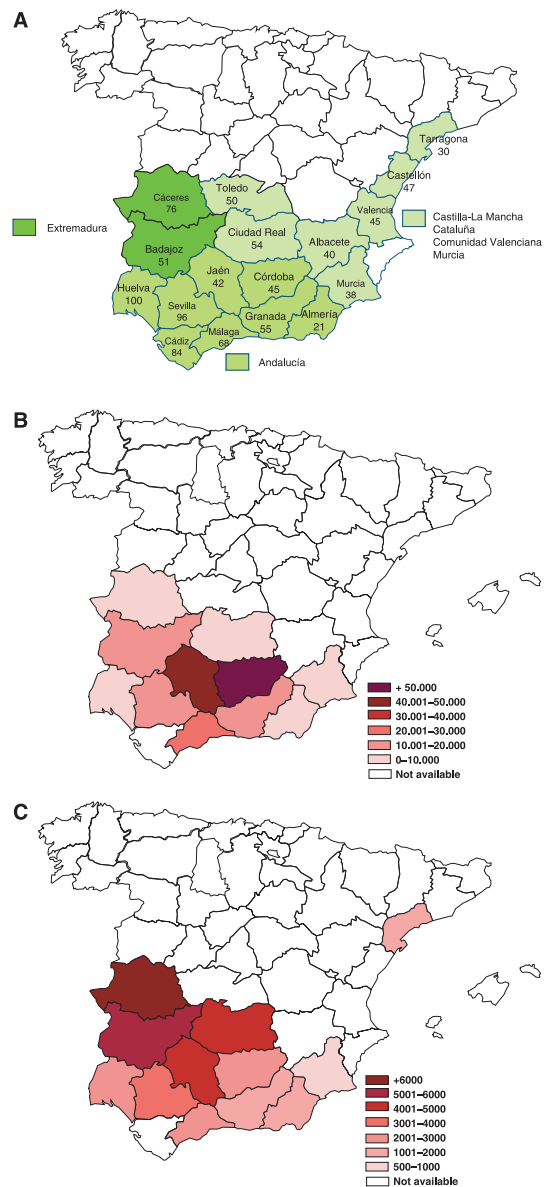


Figure 1. (A) Areas in Spain covered in the study. The study area is highlighted in green. The names of the provinces and regions mentioned in the study are shown together with the number of patients included per province. (B) Total accumulated olive pollen counts in the studied area. (C) Total accumulated grass pollen counts. Data from Red Española de Aerobiología (REA) in Boletín 4 (1998).

there are stepwise pollen gradients in the territory we studied, making it an appropriate model to analyse the influence of exposure levels and sensitization profiles as well as the relationship between pan-allergens and different pollen gradients.

Patients

About 50 patients were included per province (Fig. 1). Patients were selected consecutively during a 3-month period outside the pollen season. All patients with a compatible clinical history of pollinosis

with no previous immunotherapy and who had resided in the same location during the previous five consecutive years or longer were included in the survey. To avoid bias in patient selection, neither specific IgE (sIgE) nor skin prick tests were performed before inclusion. All participants provided written informed consent and approval from the appropriate Ethics Committees was obtained.

Patient data were collected in the course of daily practice by each participating clinical group. Each patient was identified by a numeric bar coded label. Serum samples were collected from participants, identified by bar coded labels, stored at -40°C and thawed immediately before use.

Panel of purified allergens

The following allergens were included and isolated by previously described methods: *Phleum pratense* nPhl p 1 (15) and nPhl p 5 (16), *Artemisia vulgaris* nArt v 1 (17), *Olea europaea* nOle e 1 (18), nOle e 7 (19) and rOle e 9 (20, 21), *Plantago lanceolata* nPl l 1 (22), *Parietaria judaica* nPar j 2 (23), *Cupressus sempervirens* nCup s 1 (24) and *Salsola kali* nSal k 1 (25). As pan-allergens, we used Polcalcin, r-Che a 3 from *Chenopodium album* pollen (26), nonspecific LTP from peach r-Pru p 3 (27) and profilin, a mixture of two isoforms of apple profilin, r-Mal d 4 isoform A12 (GenBank Acc. No. AF129428) and isoform B4 (GenBank Acc. No. AF129427) expressed in *Escherichia coli* and purified by affinity chromatography with a poly-L-proline-Sepharose column. In addition, natural olive pollen profilin Ole e 2 was used in some tests. The allergen was purified on a poly-L-proline-Sepharose column, followed by passage through an anti-Ole e 1 affinity column to remove traces of Ole e 1.

Specific IgE determination

The level of sIgE to the different allergens was tested on the ADVIA Centaur® platform (Bayer HealthCare Diagnostics Division). The principle of the sIgE assay is based on a reverse sandwich assay and screening was performed as previously described (28).

Statistical methods

To analyse associations between qualitative variables, we used Pearson's chi-squared method when variables fulfilled the necessary criteria and Fisher's exact test when they did not. Interference from quantitative variables was analysed with Spearman's correlation coefficient and the appropriate test to verify correlation significance when quantitative levels of allergen indicators did not follow the Gaussian hypothesis. Logistics regression and Fisher's exact test were used to evaluate the risk level of suffering from lower respiratory symptoms in patients sensitized to specific allergens, as well as the association between pan-allergens and minor allergen sensitizations.

Results

Sample description

A total of 891 patients were included in the study; 13% were under 14 years of age (average age 10.8 ± 2.9 years) and 53% were female. Of the total number of patients, 42% had rhinitis, 3% bronchial asthma and 55% had both.

Sensitization profiles based on sIgE values to major allergens

Figure 2 summarizes the geographical distribution of sensitization to the major allergens of the most frequent pollen species in the area. As expected, the frequency of pollen allergic individuals sensitized to the major olive pollen allergen (Ole e 1) was very high (75.3%). Ole e 1 reactivity reached saturation in both prevalence and median IgE values in areas with intermediate olive pollen counts. Jaén where olive pollen exposure is extremely high, showed neither the highest prevalence nor the highest median sIgE values to Ole e 1.

Grass sensitization ranked second. The overall percentages of sensitization to major allergens Phl p 1 and Phl p 5 were 53.3% and 27.2% respectively. In the western provinces, the frequencies of sensitization to these allergens reached 80% and more than 50% respectively (Fig. 2). Only 1.5% of the patients were sensitized to Phl p 5, but not to Phl p 1.

The third most common allergen was Sal k 1, the main allergen of *Salsola kali* pollen (28.8%). Sensitization occurred mainly in central and eastern regions. In some areas, *Salsola* was the most frequent cause of pollinosis. The prevalence of sensitization to *Parietaria judaica* pollen, marked by Par j 2, was statistically significant only in Mediterranean provinces. Cup s 1 and Art v 1 were also important as allergen sources (14.9% and 13% respectively). A notable characteristic of *Artemisia* pollen was that the highest prevalence rates were found in distant provinces with different climates, e.g. Cáceres (western region) and Murcia (eastern region). English plantain allergy reached significant levels only in the northwestern regions, in areas where grass allergy was predominant. Patients sensitized to Pla l 1 were usually also strongly sensitized to grass.

Sensitization to minor olive allergens

As expected, the percentages of sensitization to minor olive pollen allergens (Ole e 9 and Ole e 7) were lower (10.7% and 14.4%, respectively) than to Ole e 1. However, sensitization to these minor olive allergens was statistically significantly higher in geographical areas where olive pollen exposure is higher, reaching frequencies over 35%. Figure 3 shows the prevalence and median values for Ole e 1, Ole e 7 and Ole e 9.

The association between sensitization to different allergens and the presence of asthma was statistically significant only for olive allergens (Ole e 1: $P = 0.02$, Ole e 7: $P = 0.0002$, Ole e 9: $P = 0.0007$, Table 1). In light of this finding, the next step was to analyse these three allergens separately. We observed that in patients sensitized to Ole e 1 but not to minor allergens (Ole e 7 and Ole e 9), the association between presence of asthma and sensitization to Ole e 1 was not statistically significant ($P = 0.207$). However, in Ole e 7-positive and Ole e 9-negative patients (regardless of Ole e 1 sensitization),

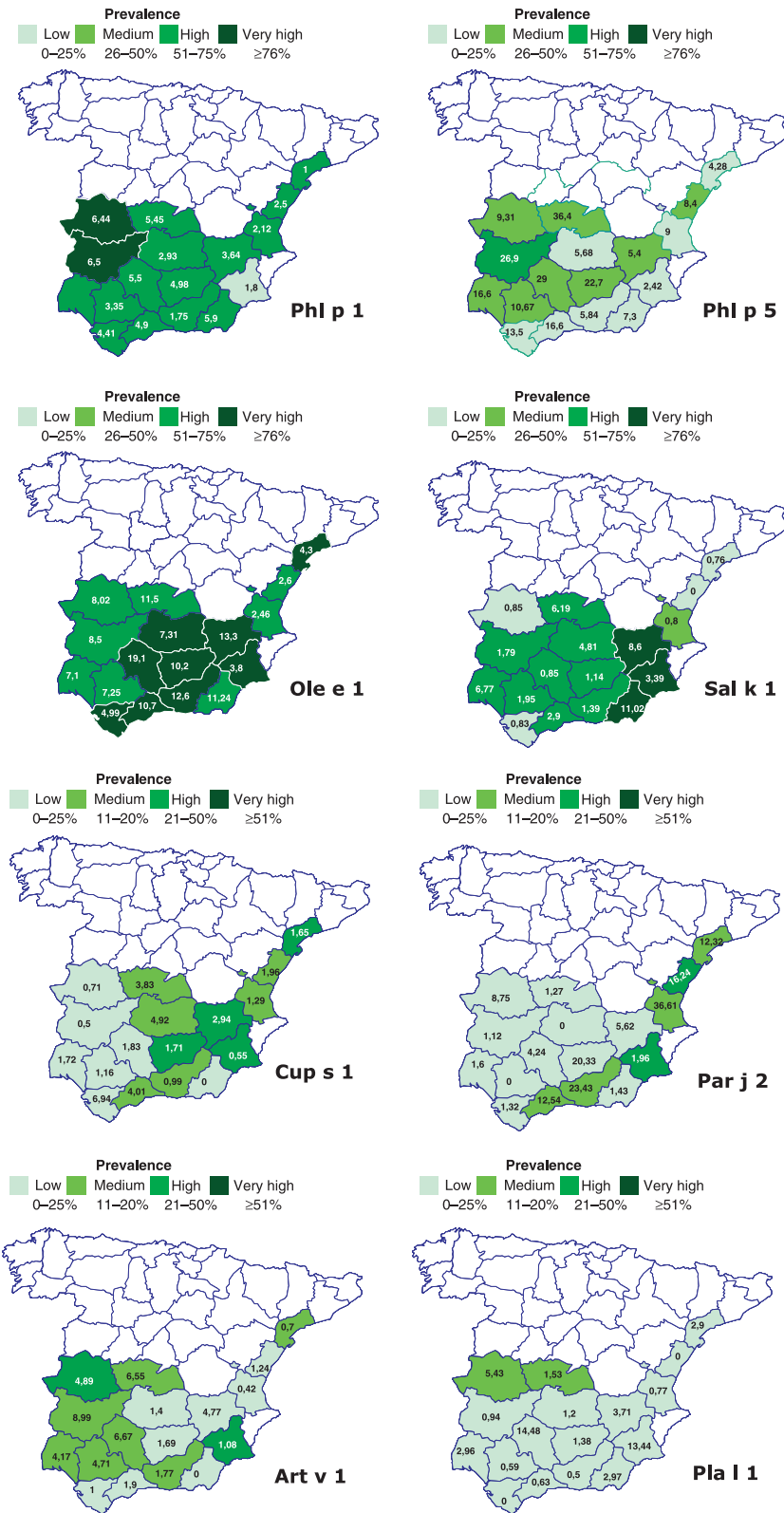


Figure 2. Prevalence of major allergen sIgE in the study area. Prevalence categories are defined as indicated, according to a colour scale. A different scale is used for the most prevalent allergens (Phl p 1, Phl p 5 and Ole e 1). Numbers inside each province indicate the median sIgE value in positive samples.

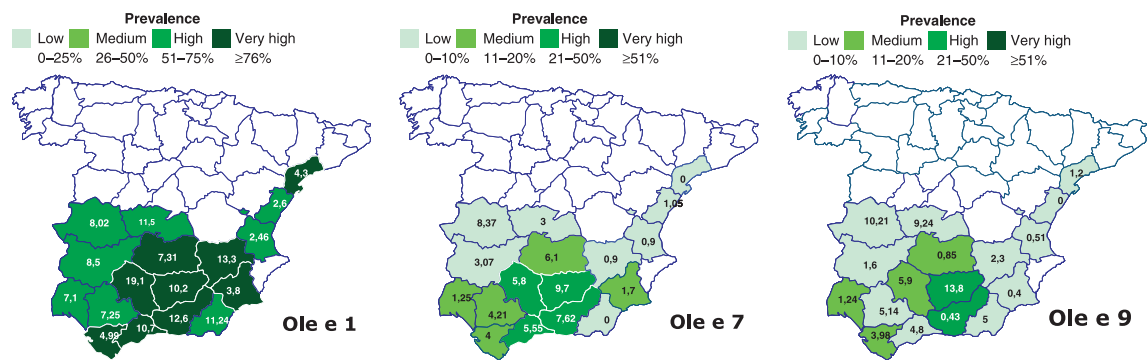


Figure 3. Prevalence of minor olive allergen sIgE (Ole e 7 and Ole e 9) in the study area compared to the prevalence of the major allergen Ole e 1. Categories are defined as indicated. Numbers indicate the median sIgE value in positive samples.

Table 1. Statistical association among different allergens and a diagnosis of asthma in the study population ($n = 891$)

	Allergen									
	Asthma									
	Positive				Negative				P-value	
	Yes	No	Yes	No	Yes	No	Yes	No		
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
Art v 1	58	59.2	40	40.8	397	57.7	291	42.3	0.8273	
Cup s 1	71	60.7	46	39.3	384	57.4	285	42.6	0.5434	
Cyn d 1	19	61.3	12	38.7	11	57.9	8	42.1	1.0000	
Ole e 9	64	75.3	21	24.7	391	55.8	310	44.2	0.0007	
Ole e 1	351	60.3	231	39.7	104	50.98	100	49.0	0.0212	
Ole e 7	86	73.5	31	26.5	369	55.2	300	44.8	0.0002	
Par j 2	32	49.2	33	50.8	423	58.7	298	41.3	0.1503	
Phl p 1	250	57.9	182	42.1	205	57.9	149	42.1	1.0000	
Phl p 5	134	59.3	92	40.7	321	57.3	239	42.7	0.6327	
Pla l 1	26	65.0	14	35.0	429	57.5	317	42.5	0.4125	
Che a 3	31	63.3	18	36.7	424	57.5	313	42.5	0.4589	
Mal d 4	69	56.6	53	43.4	386	58.1	278	41.9	0.7653	
Pru p 3	65	63.7	37	36.3	389	56.96	294	43.1	0.2370	
Sal k 1	97	64.2	54	35.8	226	56.4	175	43.6	0.1001	

the association was positive ($P = 0.012$). A similar result was obtained for Ole e 9-positive and Ole e 7-negative patients ($P = 0.046$). These results were confirmed by calculating the odds ratio (OR) for suffering asthma for each of the three allergens (Ole e 1: OR = 1.3, 95% CI: 0.94–1.81; Ole e 7: OR = 1.88, 95% CI: 1.19–2.98; Ole e 9: OR = 1.79, 95% CI: 1.04–3.1). These data indicated that the risk of having asthmatic symptoms was almost twice as high in patients sensitized to Ole e 7 or Ole e 9 as in those sensitized only to Ole e 1. As previously mentioned, the prevalence and median values for Ole e 1 seemed to reach a ceiling. Intermediate and highly exposed areas had similar values of Ole e 1 sensitization regardless of olive pollen counts. Both prevalence and median values for Ole e 7 and Ole e 9 increased along the pollen count gradient. Moreover, in highly exposed areas

(Jaén, Córdoba, Málaga and Granada), 40% of Ole e 1-negative patients were sensitized to Ole e 7, indicating that the two allergens behaved independently ($P = 0.1919$ in Andalucía). In other areas, Ole e 7 always paralleled Ole e 1 ($P = 0.03$ in Extremadura). Ole e 9 sensitization ($P = 0.015$ in Extremadura, $P = 0.002$ in Andalucía) also paralleled Ole e 1.

Sensitization to pan-allergens

Figure 4 shows the median sIgE values and prevalence for the three pan-allergens we studied. Profilin (Mal d 4) prevalence in the study area was 15%, but significant geographical variability was observed. Prevalence along the Mediterranean coast was usually below 10%, although it approached 50% in some western regions. Comparing the data in Figs 2 and 4 reveals a clear correlation between profilin and major grass allergen sensitization.

This descriptive result was confirmed when we analysed the correlation between sensitization to profilin and sensitization to other allergens (Table 2). There was a significant association between profilin and different allergens, but the magnitude of the association seemed to be higher for grass allergens. Because of significant interactions among allergens, logistic multivariate analysis was performed to predict the possible risk factors for profilin sensitization in persons also sensitized to specific allergens. The probability of being profilin-positive was clearly associated with grass allergens (Phl p 1: OR = 3.16, 95% CI: 1.71–5.83; Phl p 5: OR = 6.19, 95% CI: 3.86–9.91) and with Art v 1 (OR = 3.27, 95% CI: 1.95–5.44). When the sIgE cut-off value for the major allergen was increased to 50 kU/l, the odds of having high sIgE values for Phl p 5 was increased only in profilin-positive patients (OR = 17.70, 95% CI: 9.06–34.57).

There was no association between profilin and Ole e 1 sensitization. To check whether olive sensitization could lead to profilin sensitization in an area where exposure to olive pollen is extremely high, we analysed this area separately. The results (Table 3) revealed a relevant

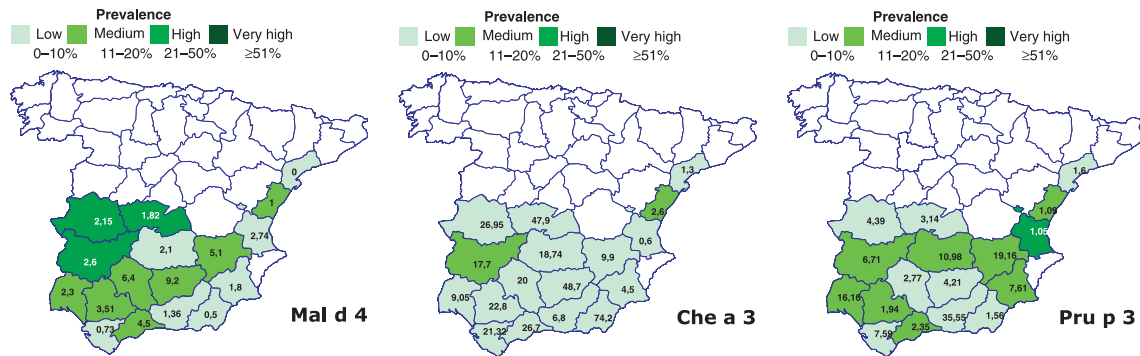


Figure 4. Prevalence of pan-allergen sIgE [profilin (Mal d 4), polcalcin (Che a 3) and LTP (Pru p 3)] in the study area. Prevalence categories are defined as indicated, according to a colour scale. Numbers indicate the median sIgE value in positive samples.

Table 2. Association between profilin (Mal d 4) and other allergens in the entire patient sample ($n = 891$)

Mal d 4 +	% patients	<i>P</i> -value
Art v 1 +	33.6	<0.0001
Art v 1 -	12.3	
Ole e 9 +	25.3	0.0057
Ole e 9 -	13.8	
Phl p 1 +	24.8	<0.0001
Phl p 1 -	3.9	
Phl p 5 +	39.3	<0.0001
Phl p 5 -	6	
Pla l 1 +	52.3	<0.0001
Pla l 1 -	13.1	
Che a 3 +	36.5	<0.0001
Che a 3 -	13.7	
Pru p 3 +	22.3	0.0329
Pru p 3 -	14	
Sal k 1 +	28.3	<0.0001
Sal k 1 -	10.3	

Fisher's exact test; only statistically significant associations are shown.

Table 3. Association between profilin and other allergens in the patient sample from the area of maximum olive exposure (Jaen, Córdoba, Granada and Málaga) ($n = 210$)

Mal d 4 +	% patients	<i>P</i> -value
Phl p 1 +	22.2	0.0001
Phl p 1 -	4.5	
Phl p 5 +	34.2	0.0001
Phl p 5 -	8.1	

Fisher's exact test; only statistically significant associations are shown.

association only between profilin and grass allergens. To rule out the possibility that olive profilin might play a different role from that of other profilins, we screened 200 pollen-allergic patients in an area of high olive pollen exposure for sensitization to both olive profilin (Ole e 2) and apple profilin (Mal d 4), the allergen used as a marker in this study. No difference in prevalence was detected and good concordance was observed (M. Lombardero, unpubl. data).

Rosaceae LTPs, marked by Pru p 3, are the main plant food allergens in the adult population of Spain (29, 30). The prevalence of Pru p 3 sensitization was significant in all areas studied here, with an average prevalence of 12.6% of the pollen-allergic population despite the fact that plant food allergy was not an inclusion criteria. This prevalence was twice as high in the paediatric subsample compared to the adult (22% vs 11% respectively $P = 0.0028$). There was no significant association with any major pollen allergen or with the other LTPs Ole e 7 and Par j 2, a finding consistent with the low amino acid sequence identity between them.

The overall prevalence of polcalcin sensitization (Che a 3) was low (5.8%) and showed no significant associations with major pollen allergens.

Discussion

The present study is, to the best of our knowledge, the first molecular epidemiological analysis to investigate a full panel of relevant major, minor and pan-allergens from pollen. The methodology, based on a reverse sIgE configuration assay, was specific and sensitive. Despite the use of purified allergens from natural sources, no cross-reactivity mediated by low affinity-IgE was detected. For example, some patients had sIgE titres to nOle e 1 as high as 600 kU/l and <0.1 kU/l to nPla l 1, a glycosylated homologous protein to the major olive allergen. This supports the previous findings showing the relative inability of this method to recognize low affinity IgE responses compared to conventional ones (31). The sensitivity of our method, where amounts in the range of only 10 ng of allergen per test are needed, allowed us to screen for a full range of relevant allergens purified from the natural source or as recombinant forms. The broad sensitivity range of the method from 0.1 to 400 kU/l of sIgE simplified the testing procedure. We were thus able to perform more than 23 000 IgE determinations in a sample of 891 pollen-allergic patients.

We investigated the allergen profile of pollen-allergic patients in an area where olive pollinosis plays a dominant role. The olive pollen exposure gradient in the regions we sampled is probably the broadest ever described for a specific pollen. In the same study area, a stepwise exposure gradient to grass pollen towards the western part was also seen. Other pollen gradients for Cupressus, Platanus, Salsola, Parietaria and Artemisia were also present. This offered a good opportunity to study the influence in allergy to different pollen species and the association between sensitization to pan-allergens and major allergens in pollen-allergic populations.

As expected, olive pollinosis was generally the leading seasonal allergy in the study area, although in the western part of Spain grass pollen was predominant. Olive pollen sensitization measured by Ole e 1 reactivity appeared to reach a ceiling in terms of prevalence and median IgE values in the population exposed to intermediate levels of olive pollen. In light of this finding, it would be interesting to monitor actual Ole e 1 levels instead of pollen counts to document possible correlations more accurately (32, 33).

In contrast, the prevalence and IgE levels of the minor olive allergens Ole e 7 and Ole e 9 clearly increased with pollen exposure. The fact that the risk of having asthmatic symptoms was twice as high in patients sensitized to minor allergens makes the sIgE test a clinically relevant marker of the olive allergy disease. In fact, in areas where exposure was extremely high, some patients were sensitized to Ole e 7, but not to Ole e 1; this profile can be considered a different way of being allergic to olive. Specific clinical profiles for different olive allergens can appear in areas of high exposure, and stress the need for a different approach to the standardization of olive allergy vaccines for this population (10–12, 34). We believe that this model can be extrapolated to other allergen exposure systems.

Olive pollinosis was more prevalent in the paediatric sample and we found a significant percentage of olive pollen-monosensitized patients, confirming that olive was the most frequent sensitizing pollen in the study area. Sensitization to the two pollen pan-allergens profilin and polcalcin did not parallel the olive pollen gradient. In fact, the lowest prevalence of profilin and polcalcin was found in areas where exposure to grass pollen was lower. One of the main objectives of this study was to determine whether profilin positivity was related to pollen sensitization. In Extremadura, the area with the highest exposure to grass pollen, we identified locations where the prevalence of profilin positivity was higher than 50%. In profilin-positive patients, the odds of having sIgE to Phl p 5 above 50 kU/l were more than 17 times higher than in profilin-negative individuals, supporting the idea that profilin might be considered a marker of the severity of grass allergy sensitization. An interesting hypothesis is that food allergy linked to profilin might be a natural progression of grass allergy. In a previous epidemiological survey of food-allergic patients (Red Vegetalia, unpubl. data), higher

disease severity was related to profilin food allergy. In this study, however, most of the profilin-sensitized patients did not display food allergy symptoms ($P = 0.7653$), suggesting that only a small fraction of profilin sensitized patients develop food allergy. It should be noted that Phl p 1 prevalence was twice that of Phl p 5.

Considering the data as a whole, it could be hypothesized that the development of grass allergy starts with sensitization to group 1, the most prevalent allergen, and progresses to group 5 sensitization, increased IgE levels, sensitization to profilin and the appearance of food allergy. Food allergy caused by profilin may thus be linked to the severity of grass allergy. A similar model, in which pollen sensitization leads to food allergy, has been proposed for birch pollinosis and apple allergy (35). The practical consequence of this mechanism is that testing patients for sensitization to profilin might be advisable as a marker of disease severity and as an indicator of nonspecific skin prick test responses with whole pollen extracts.

Unexpectedly, Salsola was the third most frequent cause of pollinosis in southern Spain. Sal k 1 is a recently characterized major allergen of Salsola (25), which is practically absent in other Chenopodiaceae species. The increase in Salsola allergy prevalence seems to have paralleled soil degradation and the advance of desertification linked to global warming. In semiarid southeastern areas, Salsola was the most frequent cause of seasonal allergy. In areas such as Ciudad Real (central Spain), where extensive irrigation has depleted the ground water and converted grassland into a semiarid area, a significant increase in Salsola allergy seems to have paralleled a decline in grass allergy.

Art v 1 sensitization reached significant levels in different regions. Art v 1-like proteins are present in the pollen of different Asteraceae species. In some areas such as Córdoba, sensitization to Art v 1 is related mostly to extensive sunflower cultivation, and patients have symptoms mainly during the harvest season (36). After grass pollen, sunflower pollen is the second most frequently associated with profilin and thus often causes diagnostic errors. *Artemisia* pollen counts are low, and sensitization seems to be mainly linked to close contact with the plant (occupational or indoor sensitization).

Food allergy related to lipid-transfer proteins (LTP) is the most frequent plant food allergy in adults in Spain (30). The leading LTP allergen (peach Pru p 3) was thus included in the panel tested in this study; interestingly, we found no relationship between this allergen and any pollinosis. The prevalence of peach allergy was high (average 12%) in all regions, especially considering that food allergy was not an inclusion criterion for this study. The prevalence in paediatric patients was twice as high as in adults. This supports the idea that early sensitization to Pru p 3 occurs and might be related to differential consumption patterns and diets regardless of the prevalence of pollinosis.

More studies are needed to test the new hypothesis arising from this study. In particular, the role of minor

allergens and clinical course should be monitored by molecular screening. We are planning a follow-up study in northern Spain, where grass is the dominant source of allergens, to obtain a global pollen sensitization map of the country. The analysis reported here nonetheless suggests a new way to diagnose pollinosis with potential to aid clinicians in correctly interpreting current diagnostic test results by using an appropriate combination of *in vivo* and *in vitro* allergy tests. Greater diagnostic accuracy can lead to more effective formulations of vaccines comprising only those allergens responsible for the allergic disease, and thus reducing the number of components in the formula. This would, we hope, increase the efficacy of this therapeutic option.

In conclusion, this study reports a new, more accurate way to investigate the interaction between allergen exposure and patient sensitization. Component-resolved diagnosis holds the potential to document variations in the actual sensitization profiles according to the level of exposure and to elucidate the role of pan-allergens such as profilin as markers of severity of grass pollen sensitization.

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