ORIGINAL ARTICLE

Sensitization to *Vitis vinifera* Pollen in a Wine Production Area: Identification of the Allergens Involved

González Mahave I[1], Lobera T[1], López-Matas MA[2], Blasco Á[1], Vidal I[1], Álvarez F[2], Carnés J[2]

[1]Hospital Universitario San Pedro, Logroño, Spain
[2]R&D Unit, LETI Pharma S.L.U., Tres Cantos, Madrid, Spain

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Abstract

Background: Vine cultivation is widely distributed in La Rioja, Spain (37% of all crops) and is associated with exposure of the general population to vine pollen. The aims of this study were to investigate the prevalence of sensitization to *Vitis vinifera* pollen in persons with respiratory allergy in the general population and to identify the allergens involved.
Materials and Methods: The study population comprised patients who came to the hospital between September 2019 and January 2020 with suspected respiratory allergy. All patients underwent skin prick testing with a panel of standardized aeroallergens, profilin, lipid transfer protein (LTP), and *V vinifera* pollen extract and prick-prick testing with fresh grapes. The in vitro study included specific IgE by ImmunoCap and ELISA, allergenic profile by immunoblot with individual sera from patients positive to *V vinifera* pollen extract, and 2D immunoblot with a pool of sera. The spots recognized by IgE were identified using mass spectrometry.
Results: A total of 151 patients were included. Of these, 124 were positive to some of the allergens tested. Thirty-four (27.4%) were positive to vine pollen in the skin prick tests. The serology study revealed positive results in 20 patients. Five vine pollen allergens were identified, and profilin was the most prevalent (30%). The other 4 allergens could be considered specific to this pollen.
Conclusions: Sensitization to vine pollen was frequent in the general population in a vine growing area. The clinical relevance of this finding is unknown owing to sensitization to other pollens in the vine pollen–positive patients. Five new vine pollen allergens were identified.

Key words: *Vitis vinifera*. Vine pollen allergy. 2D immunoblot. Mass spectrometry. Allergen identification.

Resumen

Antecedentes: El cultivo de la vid está ampliamente distribuido en La Rioja (37% de los cultivos), lo que supone una exposición de la población general al polen de esta planta. El objetivo de este estudio fue investigar la prevalencia de sensibilización al polen de *Vitis vinifera* en la población general con alergia respiratoria e identificar los alérgenos implicados.
Materiales y métodos: Se incluyeron en el estudio pacientes que acudieron al hospital entre septiembre de 2019 y enero de 2020 con sospecha de alergia respiratoria. A todos ellos se les realizó una prueba cutánea con el panel de aeroalérgenos estandarizados, profilina, LTP, extracto de polen de *V. vinifera* y *Prick prick* con uva. El estudio *in vitro* incluyó IgE específica mediante ImmunoCap y ELISA, perfil alergénico por inmunoblot con sueros individuales de pacientes positivos al extracto de polen de *V. vinifera* e inmunoblot 2D con un pool de sueros. Las proteínas reconocidas por la IgE fueron identificadas por espectrometría de masas.
Resultados: Se incluyeron un total de 151 pacientes. De ellos, 124 fueron positivos a algunos de los alérgenos analizados. 34 (27,4%) fueron positivos a polen de vid por prueba cutánea. 20 fueron positivos tras el estudio serológico. Se identificaron 5 alérgenos del polen de la vid, siendo la profilina el más prevalente (30%). Los otros 4 alérgenos podrían considerarse específicos de este polen.
Conclusión: Se detectó una alta sensibilización al polen de vid en la población general en una zona de viñedos. Se desconoce la relevancia clínica debido a la sensibilización a otros pólenes en los pacientes positivos a polen de vid. Se identificaron 5 nuevos alérgenos del polen de la vid.

Palabras clave: *Vitis vinifera*. Alergia al polen de vid. Inmunoblot 2D. Espectrometría de masas. Identificación de alérgenos.

Summary box

- **What do we know about this topic?**
  Few studies to date have addressed sensitization to vine pollen in the general population, and clinical relevance and allergenic composition are unknown.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  We demonstrated that sensitization to vine pollen is frequent in the general population. More frequent diagnosis of this sensitization would highlight its clinical relevance. Identification of the culprit allergens would help to reveal cross-reactivity with other allergens.

Introduction

Vines can adapt to a variety of climates and are cultivated throughout the world. In Spain, large areas of land are dedicated to vine cultivation. One of the most relevant areas is La Rioja, located in the north of Spain, where around 37% of the total agricultural crop corresponds to *Vitis vinifera* [1].

Although sensitization to grape, the fruit of *V vinifera*, has been reported, little information is available about sensitization to *V vinifera* pollen. Lipid transfer protein (LTP, 9 kDa) has been identified as the main allergen responsible for allergy to grape as a food (70% of the population), although other allergens probably remain unidentified. No allergen has been identified in *V vinifera* pollen to date, even though respiratory symptoms related to this pollen have been reported in farmers working with the *V vinifera* crop [2-4]. However, despite the massive scale of *V vinifera* culture and the large number of people working with grape, the prevalence of allergy to pollen from *V vinifera* remains unknown. Few studies have addressed general sensitization to *V vinifera* pollen [5], and only 2 studies [6,7] report *V vinifera* pollen sensitization as an occupational allergy. Therefore, little is known about the relevance of *V vinifera* pollen in patients attending allergology units.

V vinifera belongs to the Vitaceae family, which is the only family of the order Vitales. Most plants in this family are found in tropical areas. The only species reported to be allergenic within this family were of the genus *Vitis*, all of them as food allergens. *V vinifera* pollinates from May to June, coinciding with other allergenic pollens in our area. Despite not being botanically related, cross-reactivity between vine pollen and *Olea europaea*, *Lolium perenne*, and *Salsola kali* pollens has been described in a case report study [8].

The aims of the present study were to investigate the prevalence of sensitization to *V vinifera* pollen in La Rioja in members of the general population with respiratory allergy and to identify the allergens responsible.

Materials and Methods

Patient Population

A prospective study was performed between September 2019 and January 2020 in Hospital San Pedro, Logroño (La Rioja), Spain. Patients attending the hospital's allergology outpatient clinic with suspected respiratory allergy, rhinoconjunctivitis, and/or asthma were included. Patients with other allergies (eg, drug allergy) without respiratory symptoms were included as controls.

The study was approved by the Ethics Committee of Hospital San Pedro (study number PI 530). All patients gave their oral consent to participate. For patients aged under 18, a parent and/or the patient's legal guardian approved their participation.

Extract Manufacturing

V vinifera pollen (Iberpolen) was defatted with acetone and extracted with phosphate-buffered saline (PBS) according to internal manufacturing procedures (LETI Pharma S.L.U.). In short, vine pollen underwent 2 consecutive extraction processes lasting 4 hours and 18 hours at 4°C followed by centrifugation and collection of the supernatant. The extract was then dialyzed, filtered, frozen, and freeze-dried. The protein content was measured using the Bradford method (Pierce Biotechnology).

Nonstandardized skin prick tests (SPTs) were prepared at a concentration of 2 mg of freeze-dried material/mL, which corresponds to a concentration of 385 µg of protein/mL.

Skin Prick Tests

SPTs were performed on the volar surface of the forearm using standardized lancets in all patients included in the study according to the method reported by the Global Allergy and Asthma European Network (GALEN) on Skin Tests of the European Academy of Allergology and Clinical Immunology [9]. A panel with various biologically standardized allergens (LETI Pharma) was used and included mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), molds (*Alternaria alternata*), animal epithelium (cat and dog), pollens (*Phleum pratense*, *Secale cereale*, *Artemisia vulgaris*, *Parietaria officinalis*, *O europaea*, *Plantago lanceolata*, *Betula alba*, *Corylus avellana*, *Chenopodium album*, *S kali*, *Fraxinus excelsior*, *Quercus ilex*, *Platanus acerifolia*, *Populus nigra*, *Cupressus arizonica*, *Pinus radiata*, and *V vinifera*), profilin (purified Pho d 2 from *Phoenix dactylifera*), and LTP (Pru p 3) (LETI Pharma). Prick-by-prick testing with fresh grapes was also performed. Histamine (10 mg/mL) and negative solutions were used as controls.

A control group with no respiratory disease was tested with *V vinifera* pollen, profilin (LETI Pharma), LTP (LETI Pharma), and grape.

Serum samples were collected from patients with positive results who gave their consent. Patients who were positive to vine pollen were asked about the area of residence and the duration of the clinical course of the respiratory symptoms (years).

Protein Profile (SDS-PAGE and 2D Electrophoresis)

The protein profile of the extracts was investigated using SDS-PAGE. Briefly, 10 µg of protein from the *V vinifera* extract was loaded in SDS-PAGE gels (2.67% C, 15% T acrylamide) under reducing conditions and stained with Oriole (Bio-Rad Laboratories). The molecular weight (MW) of the proteins was calculated using ImageQuantTL software version 8.1 (Cytiva).

The protein profile was also analyzed using 2D electrophoresis. The extract was purified and concentrated with ammonium sulfate in 2 steps. The first step was performed at 40% ammonium sulfate saturation, the pellet was stored at 4°C after centrifugation, and the supernatant was precipitated with ammonium sulfate at 80% and maintained at 4°C overnight. Thereafter, the sample was centrifuged, and the pellet was collected, reconstituted in ultra-purified water, and mixed with the pellet from the 40% precipitation. The concentrated extract was cleaned with the ReadyPrep 2-D Cleanup Kit (BioRad). The proteins were separated according to their isoelectric point (pI) into ReadyStrip IPG Strips (BioRad) in a pH range of 3-10 using Protean IEF Cell (BioRad). After the first dimension, the strip was equilibrated with ReadyPrep 2-D Cleanup Kit buffers (Bio-Rad), and proteins were separated in the second dimension according to their MW. The spots were developed with Oriole fluorescent gel stain (BioRad), and the image was captured with an Amersham Imager 6 device (Cytiva).

Specific IgE (CAP)

Serum specific IgE (sIgE) for each allergen with positive results in SPT was analyzed in all patients using ImmunoCAP (Thermo Fisher Scientific). rPhl p 1, rPhl p 5, rPru p 3, and rPhl p 12 were also investigated.

A total of 2.1 mg of protein from *V vinifera* pollen extract was labeled using a biotin kit (Roche Diagnostics). Aliquots of 50 µL of biotin-labeled *V vinifera* extract were incubated in streptavidin ImmunoCAPs (Thermo Fisher Scientific) for 30 minutes, and the assay continued as with the commercial ImmunoCAPs. The experiment was performed using the ImmunoCAP 100E system (Thermo Fisher Scientific).

sIgE (ELISA)

Ten micrograms of *V vinifera* protein per well was used to coat Immulon 4 HBX microplates (Thermo Fisher Scientific). Each serum sample (diluted 1:1 with PBS) was added to the plate and incubated for 2 hours at room temperature. After 3 washes with PBS-0.1% Tween, peroxidase-conjugated monoclonal antihuman IgE (Southern Biotech) (dilution 1:20 000) was added. After 2 hours, the reaction was developed with 3,3',5,5'-tetramethylbenzidine, stopped with 0.16 M sulfuric acid, and read at 450 nm using a plate reader (Thermo Fisher Scientific). Serum with an optical density equal to or below 0.03 was considered negative (3 times the value of the negative control).

Allergenic Profile

The allergenic profile was investigated using immunoblot of the individual sera. Briefly, after SDS-PAGE or 2D electrophoresis of *V vinifera* extract, proteins were electrotransferred onto a Trans-Blot Turbo Transfer Pack (Bio-Rad) and dried at room temperature. Thereafter, membranes were incubated overnight with the individual sera diluted 1:4 in PBS. After incubation with monoclonal antihuman-IgE-PO (Southern Biotech) the reaction was developed with Clarity Western ECL Substrate (Bio-Rad) and visualized using chemiluminescence. A serum pool was prepared with identical amounts from the 17 individuals for whom bands in the immunoblot were recognized. This pool was used to identify the positive spots in a 2D immunoblot.

Allergen Sequencing

Spots recognized in the 2D immunoblot were excised from the gel, digested with trypsin, sequenced, and identified using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS) at the Proteomic Unit of Complutense University, Madrid, Spain.

Statistical Analysis

Descriptive statistical analyses were used to investigate variables. The Fisher exact test was used to compare *V vinifera*–positive and –negative individuals. The analysis was performed using GraphPad Prism 9.1.0 software (GraphPad Software).

Results

Patient Population

The study population comprised 151 patients (89 females [58.9%]; mean [SD] age, 33.7 [18.0] years [range, 4 to 76 years]) with respiratory symptoms and 30 controls (24 females [80%]; age, 49.8 [19.6] years [range, 10 to 87 years]). Patients had a previous history of rhinitis/rhinoconjunctivitis (50.9%), asthma (17.6%), or rhinitis and asthma (31.4%).

Skin Prick Test

Positive SPT results to any of the allergenic sources tested were recorded in 124 patients. Twenty-seven patients with rhinitis (17.9%) and 30 controls without respiratory symptoms had negative SPT results to all the allergens tested.

The sensitizations of patients with positive results are shown in Table 1. The most prevalent allergen was grass pollen, which affected 66.9% of those with a positive result, and the least prevalent was *A alternata*, with 5.6%. Patients sensitized to *V vinifera* pollen were also sensitized to other pollens, as well as to the panallergens profilin and LTP. These associations were statistically significant ($P<.05$).

Table 1. Sensitization of the Study Population.

	Total positive	*Vitis vinifera*–negative	*Vitis vinifera*–positive	*P* Value (Fisher exact)
No.	124	90 (72.6%)	34 (27.4)	
Mites				
D pteronyssinus	35 (28.2%)	26 (28.9%)	9 (26.5%)	NS
D farinae	27 (21.8%)	22 (24.4%)	5 (14.7%)	NS
Molds				
A alternata	7 (5.6%)	3 (3.3%)	4 (11.7%)	NS
Epithelia				
Dog	25 (20.2%)	17 (18.9%)	8 (23.5%)	NS
Cat	28 (22.6%)	19 (21.1%)	9 (26.5%)	NS
Pollen				
P pratense	83 (66.9%)	54 (60.0%)	29 (85.3%)	.0096
S cereale	80 (64.5%)	51 (56.6%)	29 (85.3%)	.0031
A vulgaris	18 (14.5%)	8 (8.9%)	10 (29.4%)	.0080
P officinalis	13 (10.5%)	5 (5.6%)	8 (23.5%)	.0069
O europaea	57 (46.0%)	28 (31.1%)	29 (85.3%)	<.0001
P lanceolata	56 (45.2%)	33 (36.6%)	23 (67.6%)	.0025
B alba	22 (17.7%)	8 (8.9%)	14 (41.5%)	<.0001
C avellana	11 (8.9%)	3 (3.3%)	8 (23.5%)	.0014
C album	48 (38.7%)	24 (26.7%)	24 (70.6%)	<.0001
S kali	30 (24.2%)	16 (17.8%)	14 (41.5%)	.0097
F excelsior	47 (37.9%)	22 (24.4%)	25 (73.5%)	<.0001
Q ilex	12 (9.7%)	5 (5.6%)	7 (20.6%)	.0182
P acerifolia	31 (25.0%)	15 (16.7%)	16 (47.1%)	.0009
P nigra	18 (14.5%)	7 (7.8%)	11 (32.4%)	.0012
C arizonica	24 (19.4%)	9 (10.0%)	15 (44.1%)	<.0001
P radiata	2 (1.6%)	1 (1.1%)	1 (2.9%)	NS
Molecular allergens				
Profilin	13 (10.5%)	6 (6.7%)	7 (20.6%)	.0433
LTP	9 (7.3%)	1 (1.1%)	8 (23.5%)	.0001
Grape	9 (7.25%)	1 (1.1%)	8 (23.5%)	.0001

Abbreviation: NS, nonsignificant.

V vinifera–Positive Patients

Of the 34 *V vinifera*–positive patients, 18 were women (52.9%) with a mean age of 29.6 (16.8) years (range, 8 to 76). Five were children aged below 10 years and 7 were adolescents aged between 11 and 20 years.

None of the 34 *V vinifera*–positive patients were monosensitized. Most were sensitized to other pollens, such as grasses (85.3%), *O europaea* (85.3%), *Chenopodiaceae* (70.6%), and/or *P lanceolata* (67.7%), as well as to mites (26.7%), *A alternata* (11.7%), and animal epithelium (26.5% to cat and 23.5% to dog).

The clinical course of respiratory symptoms lasted 5.7 (4.0) years, varying between 1 year (4 cases) and 16 years (1 case). The clinical course of respiratory symptoms lasted less than 3 years, 4 to 7 years, and more than 8 years in 14, 9, and 11 patients, respectively.

Twenty-two patients had rhinitis (64.7%). Two had bronchial asthma (5.8%), and 10 had rhinoconjunctivitis (29.4%); of these, 7 were from urban areas and 5 were from semirural areas. Fourteen of the patients sensitized to *V vinifera* lived in a semirural area (41.2%). This number decreased in patients with negative results for pollen (21.4%) and was 35.4% in patients with positive results only for pollen (Table 2).

Table 2. Demographic and Clinical Characteristics of the Population.[a].

	Cases				Controls
Skin prick test	***Vitis vinifera* +**	**Pollen –**	**Pollen +**	**Negative**	
No.	34	28	62	27	30
Mean (SD) age, y	29.6 (16.8)	33.2 (19.4)	32.2 (13.8)	43.6 (23.1)	49.8 (19.6)
Female sex, %	63.1	66.7	50.0	89.7	80
Semirural residence, %	41.2	21.4	35.4	22.2	34.8
Respiratory symptoms					
Rhinitis, %	64.7	66.2	35.7	74.1	NA
Asthma, %	5.8	3.1	32.1	22.2	NA
R-A, %	29.4	30.8	32.1	7.4	NA
Mean (SD) clinical course	5.7 (4.0)	7.3 (7.2)	8.1 (5.8)	NA	NA

Abbreviation: R-A, rhinitis and asthma.
[a]Patients were selected by skin prick test result. The negative column included patients who were negative to all the allergens tested.

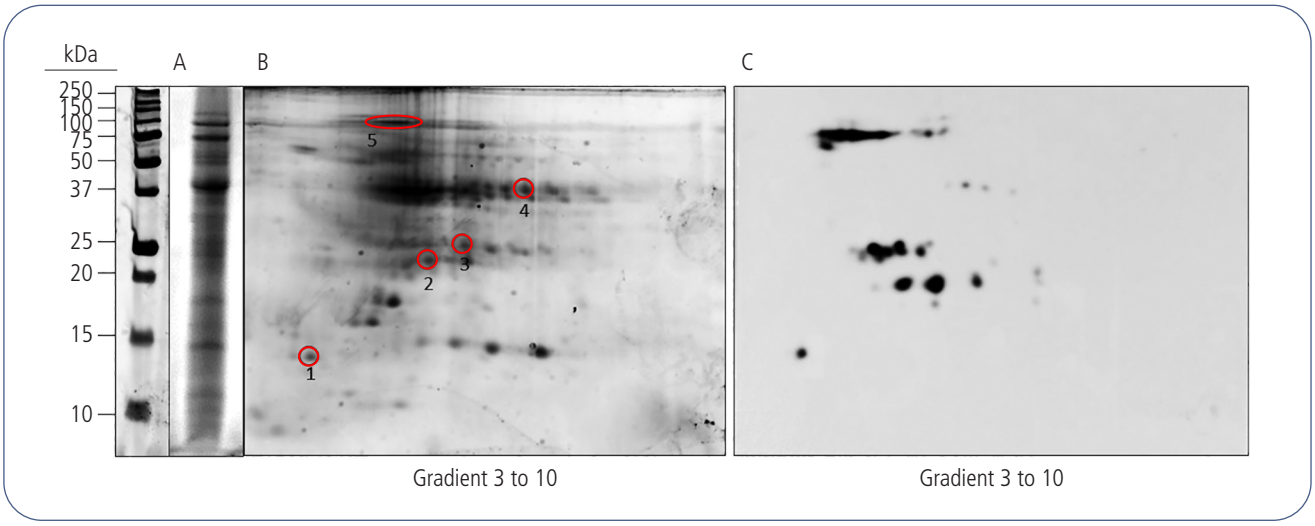

Figure 1. Protein and allergenic profile of *Vitis vinifera* pollen extract. A, SDS-PAGE of *V vinifera* pollen extract (10 µg of protein). B, 2D electrophoresis using a pH gradient from 3 to 10. Both gels (A and B) were stained with Oriole. C, 2D immunoblot showing binding of IgE from pooled sera to *V vinifera* pollen extract. Spots chosen for protein identification by liquid chromatography mass spectrometry/mass spectrometry are marked in red in panel B.

Eight patients (23.5%) were also sensitized to grape, and none of them presented symptoms caused by ingestion of this fruit. Eight patients (23.5%) were sensitized to LTP and 7 (20.6%) to profilin.

None of the patients included in the control group were positive for these allergens.

Protein Profile and 2D Analysis

The SDS-PAGE findings for the *V vinifera* pollen extract are shown in Figure 1A. Several bands were observed between 10 and 100 kDa. The 38-, 64-, and 82-kDa bands were the most prominent.

In 2D electrophoresis, the MW of the proteins concurred with those reported in SDS-PAGE. The distribution of the isoforms separated by the pI has a pH range of between 3 and 8. The 15-, 25-, and 38-kDa bands presented at least 4 isoforms. Some bands were observed more clearly in 2D than in SDS-PAGE, especially the 20- and 25-kDa bands (Figure 1B).

Specific IgE

Sera from 33 of the 34 *V vinifera* pollen–positive patients were obtained. Sixteen (48.5%) were positive to *V vinifera* by ImmunoCAP, 3 with sIgE levels below the generally accepted threshold (0.35 kU/L). Fourteen (42.4%) were positive by ELISA.

Allergenic Profile

Seventeen individual serum samples (51.5%) recognized different proteins in the immunoblot (Figure 2).

A total of 20 individuals (60.6%) were positive to any of the 3 methods used to detect sIgE. Most (12 [60%]) were positive

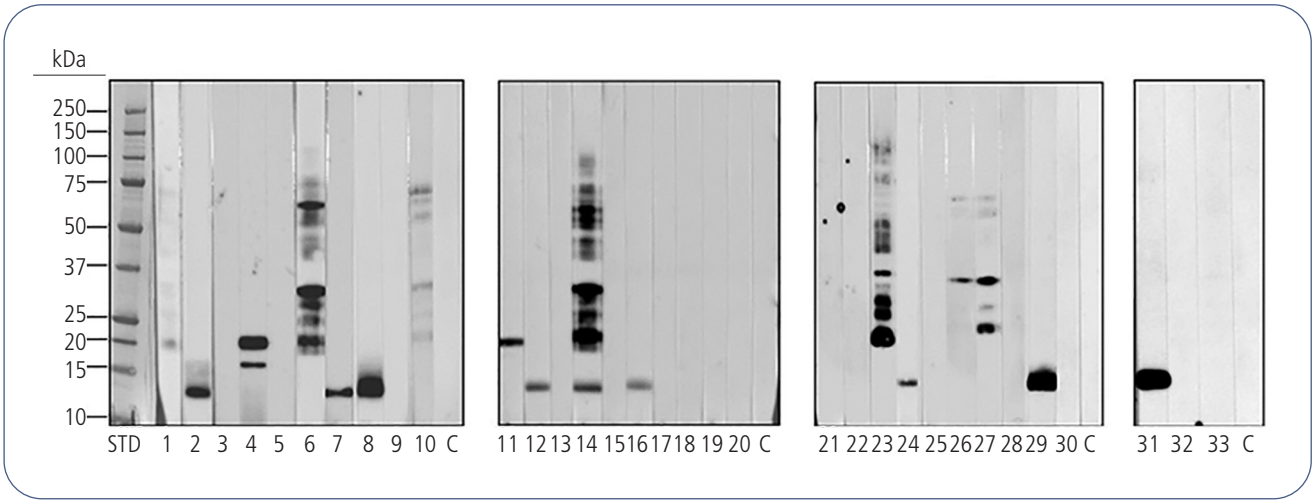

Figure 2. Allergenic profile. Immunoblot with individual sera from 33 patients with positive skin prick test results for *Vitis vinifera* pollen. Twenty micrograms of protein from *V vinifera* extracts was used in each lane; individual sera were diluted 1/5.

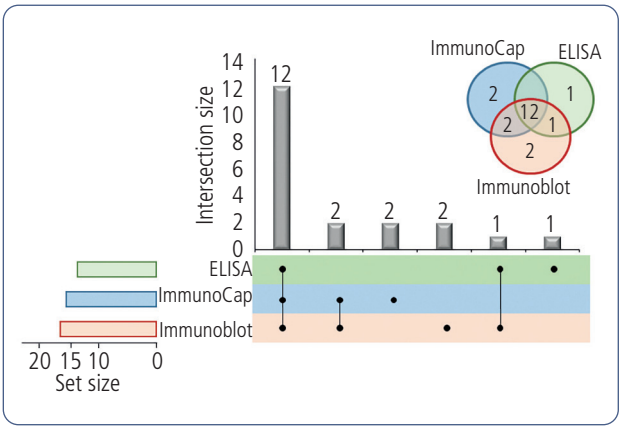

Figure 3. UpSet plots and Venn diagram representing the patients with positive sIgE for *Vitis vinifera* by the different techniques used in the study.

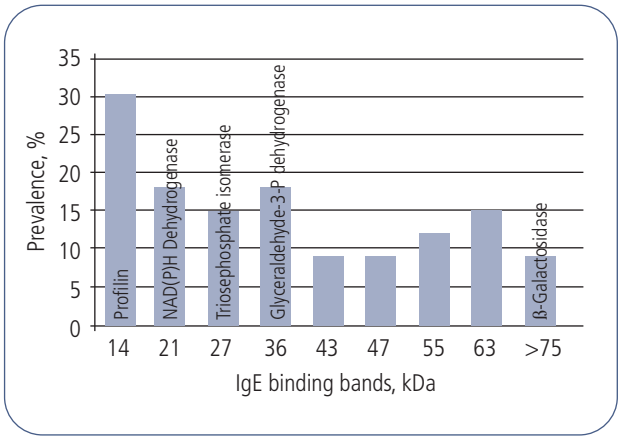

Figure 4. Allergogram. Percentage of patients recognizing each sIgE binding band. Potential identification of the *Vitis vinifera* allergens are included in the graph.

with all 3 methods: ImmunoCAP, ELISA, and immunoblot (Figure 3).

The MW and number of patients who recognized each band were 14 kDa in 10 patients (30.3%), 21 and 36 kDa in 6 patients (18.2%), 27 and 63 kDa in 5 patients (15.2%), and 55 kDa in 4 patients (12.1%). Bands of 43 kDa and 47 kDa and bands higher than 75 kDa were detected in 3 patients (9.1%). This distribution can be observed in Figure 4.

The pool of sera recognized spots in 5 areas of the 2D immunoblot (Figure 1C). Four of these spots contain various isoforms with different pIs. The spots recognized by the IgE were approximately 14, 21, 27, 38, and higher than 75 kDa.

Cross-reactivity with other pollens was studied using immunoblot inhibition (Supplementary material figure S1). The 14-kDa band was inhibited by other pollen extracts. The extract was totally inhibited by *P pratense* extract and partially inhibited by *O europaea*. In the case of *S kali*, *P lanceolata*, *C album*, and *C arizonica*, only the 14-kDa allergen was inhibited.

Allergen Identification

The identification of the 5 spots recognized by the IgE (Figure 1B) in the 2D immunoblot using LC-MS/MS analysis is shown in Table 3. These protein bands were identified as allergens in *V vinifera* and corresponded to profilin (14.2 kDa), NAD(P)H dehydrogenase (21.7 kDa), triosephosphate isomerase (27.1 kDa), glyceraldehyde-3-phosphate dehydrogenase (36.5 kDa), and ß-galactosidase (92.9 kDa). Profilin identity was confirmed by cross-reactivity with purified Pho d 2 (Figure S2, Supplementary material). Together with ß-galactosidase, spot 5 contained an uncharacterized protein. The allergenic prediction was nonallergen (AlgPred: http://crdd.osdd.net/raghava/algpred/index.html). Therefore, and since the IgE recognition area in spot 5 was diffuse and a larger area was cut for the LC-MS/MS analysis (Figure 1B), this uncharacterized protein was ruled out as a vine allergen.

Table 3. *Vitis vinifera* Pollen Allergens Identified by Liquid Chromatography Mass Spectrometry/Mass Spectrometry.[a].

Spot	Accession Number	Protein name	% Coverage	Peptide matches	Theoretical MW (kDa)
1	A5ASF9 and A5BLM8	Profilin	66	9	14.2
MSWQTYVDDHLMCEIDGQGQHLTAAAIVGHDGSVWAQSTSFPEFK**TPEITGIMNDFAEPGHLAPTGLYLGGTKYMVIQGEPGAVIRGKKGSGGITIKKTGQ ALVFGIYEEPVTPGQCNMVVERLGDYLVDQGL**					
2	A5AS18	NAD(P)H dehydrogenase	58	13	21.7
MVTKVYIVYYSMYGHVEK**LAEEIKKGAASVEGVEAKLWQVPETLPEEVLGK**MSAPPK**SDTPIITPTDLAEADGFVFGFPTRFGMMAAQFKAFLDATGGLWR** TQQLAGKPAGIFYSTGSQGGGQETTALTAITQLVHHGMIFVPIGYTFGAGMFEMEK**VKGGSPYGAGTFAGDGSRQPSELELEQAFHQGKYIAGITKK**LKEAA					
3	D7TLU7	Triosephosphate isomerase	74	14	27.1
MGRK**FFVGGNWK**CNGTGEEVKK**IVSTLNAGEVPSGDVVEVVVSPPFVFLPLVKSTLRPDFHVAAQNCWVK**KGGAFTGEISAEMLVNLGIPWVIIGHSER**RLL LNESNEFVGEKVAYALSKGLKVIACVGETLEQRESGSTMEVVAAQTKAIADKVSNWANVVLAYEPVWAIGTGKVATPAQAQEVHSELR**NWFQANASPE VAATIR**IIYGGSVSGANCKELAAKPDVDGFLVGGASLKPEFIDIIK**SAEVKKNC					
4	A0A438H737	Glyceraldehyde-3-phosphate dehydrogenase	74	28	36.5
MAKIKIGINGFGRIGRLVARVALQRDDVELVAVNDPFINTDYMTYMFK**YDSVHGQWK**HHDIK**VKDSKTLLFGDKAVTVFGAKNPEEIPWGEAGAEYVVESTGV FTDKDK**AALHLKGGAKK**VIISAPSSNAPMFVVGVNEKEYKSNIDIVSNASCTTNCLAPLAKVIHDKFGIVEGLMTTVHSITATQKTVDGPSMKDWR**GGR AASFNIIPSSTGAAKAVGK**VLPALNGK**LTGMAFRVPTADVSVVDLTVRTEKKASYDDIKAAIKAESEGNLKGILGYTEDEVVSSDFLGDSRSSIFDAKAGIA LNENFIKLVSWYDNEWGYSSR**VVDLIRHIDSTK					
5	D7TCB5	Beta-galactosidase	31	27	92.9
MYRTYFLGNTSVASSKNATHAISFCVLFVLLNVLASAVEVSYDGRALIIDGKR**RVLQSGSIHYPRSTPEMWPDLIR**KAKAGGLDAIETYVFWNVHEPLRR**EYDFSGNL DLIRFIQTIQAEGLYAVLR**IGPYVCAEWTYGGFPMWLHNMPGIEFRTANKVFMNEMQNFTTLIVDMAKQEK**LFASQGGPIIIAQIENEYGNIMAPYGDAGK**VYV DWCAAMANSLDIGVPWIMCQQSDAPQPMINTCNGWYCDSFTPNNPNSPK**MWTENWTGWFK**NWGGKDPHR**TAEDLSYSVAR**FFQTGGTFQNYYMYHGGTNF GR**VAGGPYITTSYDYDAPLDEFGNLNQPK**WGHLKDLHTVLKSMEETLTEGNITTIDMGNSVEVTVYATQKVSSCFFSNSNTTNDATFTYGGTEYTVPAWSVSILPDCK KEVYNTAK**VNAQTSVMVKNKNEAEDQPASLKWSWRPEMIDDTAVLGK**GQVSANRLIDQKTTNDRSDYLWYMNSVDLSEDDLVWTDNMTLRVNATGHILHA YVNGEYLGSQWATNGIFNYVFEEKVKLKPGKNLIALLSATIGFQNYGAFYDLVQSGISGPVEIVGR**KGDETIIKDLSSHK**WSYKVGMHGMAMK**LYDPESPYK**WEEG NVPLNRNLTWYKT**TFKAPLGTDAVVVDLQGLGK**GEAWVNGQSLGRYWPSSIAEDGCNATCDYRGPYTNTKCVRNCGNPTQRWYHVPRSFLTADENTLVLFEEFGG NPSLVNFQTVTIGTACGNAYENNVLELACQNRPISDIKFASFGDPQGSCGSFSK**GSCEGNKDALDIIKKACVGKESCSLDVSEKAFGSTSCGSIPKR**LAVEAVC					

Abbreviation: MW, molecular weight.
[a]Peptides identified in the assay are marked in bold in the corresponding amino acid sequence.

Discussion

Sensitization to pollens is influenced by the geographical area where the patient lives. However, some pollens are not common sensitizers but could cause allergy in high-exposure areas. Such would be the situation of geographical areas near specific types of crops, such as grapes, with large areas dedicated to the growth of *V vinifera* and, therefore, with high exposure to its pollen. We studied the prevalence of pollen from *V vinifera* in a high-exposure area and identified the 5 most relevant allergens that are responsible for sensitization.

To date, only a few cases of sensitization to vine pollen have been reported [5,7,8,10]. In this study, sensitization to *V vinifera* pollen was detected in a high percentage of patients with allergic respiratory disease, ie, 34 patients out of 124 with pollen allergy (27.4%). The sensitization detected in the general population is higher than in other studies—9% in the study by Feo et al [5] and 14% in that by Perontin et al [7]—probably in association with high exposure to this crop in the region, suggesting that the general population can be sensitized without occupational exposure that would favor said sensitization. Consistent with these observations, we found that a higher percentage of vine pollen–sensitized patients (58.8%) live in urban areas, thus supporting the observation that further exposure is not required or there is no need to be in a professional setting for sensitization to vine pollen. This also leads us to suppose that vine pollen may be more airborne than previously thought [10] or that by not attaining high environmental levels, it might have a high capacity for sensitization. Calculation of vine pollen levels in our area would help to explain more precisely or resolve this possibility.

All patients sensitized to *V vinifera* pollen were also sensitized to other pollens, mainly grasses, *O europaea*, and weeds (*P lanceolata* and Chenopodiaceae). Cross-reactivity between *V vinifera* and *P pratense* and partial inhibition with other pollens, mainly *O europaea*, was revealed by immunoblot inhibition. Cross-reactivity between *V vinifera* and other pollens has been reported [5,8]. Profilin was inhibited by other pollen extracts; therefore, in patients exclusively sensitized to profilin, positivity for *V vinifera* could be due to cross-reactivity with other pollens. Against this backdrop, a sensitization sequence has been reported for grass pollen proteins, relating profilin to the progress of sensitized patients over time [11]. In our study, only 7 patients were sensitized to profilin (9 by immunoblot), suggesting that most of the patients in the present study were sensitized earlier to vine pollen than to other pollens. In addition, the duration of the clinical course in this group of patients (5.7 years) is lower than in patients

sensitized exclusively to other pollen groups (8.1 years) or to other allergens (7.3 years), suggesting an association with primary sensitization to vine pollen in addition to that related to the clinical course.

A previous publication reported sensitization to vine pollen and grape simultaneously and cross-reactivity between the allergenic structures of vine pollen and grape [8]. In our study this possibility was ruled out, as only 8 patients (23.5%) in the *V vinifera* pollen–positive population were sensitized to grape, and all of them presented good tolerance after intake.

Our study is limited by the determination of the clinical relevance of *V vinifera* pollen sensitization. In all cases, sensitization to grasses and *O europaea* pollen was detected. The pollination period of these plants matches that of *V vinifera*, which flowers at the end of May or early June [10]. Additionally, since the atmospheric concentration of *V vinifera* pollen in the area is unknown, no relationship can be established between pollen levels and symptoms. Previous studies of vineyard workers with occupational allergy showed that, except in specific cases [10], symptoms were produced by grass pollen [7], with vine pollen having no clinical relevance [12]. However, it should not be ruled out that symptoms produced by vine pollen may be masked because of co-occurrence with the symptoms caused by other, more allergenic pollens and with higher concentrations [5,10]. In these studies, the authors investigated clinical relevance using bronchial and/or ocular provocation testing and confirmed the increase in respiratory symptoms in areas close to the vineyards, where individuals were more likely to be exposed to vine pollen. A similar analysis should be performed in the present study population to determine clinical relevance.

Only 1 study examined the allergens at issue [5]. In this case, the MW was 45 and 67 kDa. We also observed IgE-binding bands with these MW. However, they were not the most prevalent and were not detected in the 2D immunoblot with the pool of sera.

The allergens involved in *V vinifera* sensitization were investigated in-depth, and 27.2% of patients were found to be sensitized to profilin. Among the allergens identified, profilin is a well-known panallergen that is highly cross-reactive with plant allergens [13]. We also demonstrated this cross-reactivity by in vitro inhibition of *V vinifera* profilin with Pho d 2.

However, another 4 allergens were also detected. NAD(P)H dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were recognized by 18% of patients. Allergens from the NAD(P)H dehydrogenase family have been reported in molds (Alt a 7 and Cla h 7) [14]. Their amino acid sequences share approximately 50% identity with the allergen found in vine. Glyceraldehyde-3-phosphate dehydrogenase was reported in wheat as an occupational allergen in baker's asthma [15]. It corresponds to the allergen Tri a 34 and has a sequence identity of 84.6% compared with the vine allergen. A protein homologous to Tri a 31 (triosephosphate isomerase) was recognized by 15% of patients. Allergen from the triosephosphate isomerase family has been reported in seafood, mites, watermelon, and wheat (Tri a 31 [14-16]). Wheat has a sequence identity of 79.5% compared with vine allergens. Finally, ß-galactosidase was also recognized. This has been identified as an allergen in some Mediterranean trees such as olive and cypress. Its allergenic properties and its role in cross-reactivity have been reported [17]. The only ß-galactosidase pollen included in the Allergome database (Pho d 90 kD) corresponds to a palm tree allergen [18]. These 4 allergens could be considered specific to *V vinifera* pollen.

In summary, we detected significant sensitization to vine pollen in the general population living close to vineyards. The clinical significance of our findings is limited, as all patients were also sensitized to other, more allergenic pollens that pollinate at the same time. We report 5 novel vine allergens in a sensitized population. Further studies are needed to confirm their relationship with allergic symptoms, cross-reactivity with other pollens or foods, and the clinical relevance of vine pollen allergy.

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Conflicts of Interest

MA López-Matas, F Álvarez, and J Carnés are employees of LETI Pharma.

The remaining authors declare that they have no conflicts of interest.

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Idoia Gonzalez Mahave

Hospital Universitario San Pedro
Allergy Department
Calle Obispo Lepe, s/n
26004 Logroño (La Rioja), Spain
E-mail: migmahave@riojasalud.es