# Sensitization to *Vitis vinifera* pollen in a wine production area. Identification of the allergens involved

**Short title: Allergens involved in vine sensitization**

González Mahave I[1], Lobera T[1], López-Matas Mª A[2], Blasco Á[1], Vidal I[1], Álvarez F[2], Carnes J[2]

[1]Hospital Universitario San Pedro. Logroño. Spain.

[2]R&D Unit, LETI Pharma S.L.U., Tres Cantos, Madrid, Spain.

**Corresponding author:**

Idoia Gonzalez Mahave
Allergy Department.
Hospital Universitario San Pedro
Logroño. La Rioja
E-mail: migmahave@riojasalud.es.



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**Abstract**

**Background:** Vine culture is widely distributed in La Rioja (37% of the crops), which involves exposure of the general population to this plant's pollen. The aim of this study was to investigate the prevalence of sensitization to *Vitis vinifera* pollen in the general population with respiratory allergy and to identify the allergens involved.

**Materials and methods:** Patients who came to the hospital between September 2019 and January 2020, with suspected respiratory allergy were included in the study. All of them were skin prick tested with a panel of standardized aeroallergens, profilin, LTP, *V. vinifera* pollen extract and prick-prick with fresh grapes. *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 immunoblot 2D with a pool of sera. The spots recognized by IgE were identified by mass spectrometry.

**Results:** A total of 151 patients were included. Of them, 124 were positive to some of the allergens tested. Thirty-four (27.4%) were positive to vine pollen by skin prick test. After the serological study, 20 had positive results. Five vine pollen allergens were identified, and profilin was the most prevalent (30%). The other four allergens could be considered specific to this pollen.

**Conclusion:** A high sensitization to vine pollen was detected in the general population in a vineyard area. The clinical relevance is unknown due to the sensitization to other pollens in the vine pollen positive patients. Five new vine pollen allergens were identified.

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





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**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. Treinta y cuatro (27,4%) fueron positivos a polen de vid por prueba cutánea. Veinte fueron positivos tras el estudio serológico. Se identificaron cinco alérgenos del polen de la vid, siendo la profilina el más prevalente (30%). Los otros cuatro 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 cinco nuevos alérgenos del polen de la vid.





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

## Introduction

Vine culture is globally distributed and adapted to a wide variety of climates. In Spain there are large areas dedicated to vine cultivation. One of the most relevant is La Rioja, located in the North of Spain, where around 37% of the total agricultural crop corresponds to *Vitis vinifera* [1].

Although grape sensitization, the *V. vinifera* fruit, has been already reported, little information is available about pollen sensitization to *V. vinifera*. Only lipid transfer protein (LTP, 9 kDa) has been identified as the main allergen responsible for grape allergy as food (70% of the population), but other allergens probably remain unidentified. However, no allergen has been identified in *V. vinifera* pollen until now. This is despite respiratory symptoms related to *V. vinifera* pollen having been reported in farmers, involving the *V. vinifera* crop [2-4]. However, in spite of the massive *V. vinifera* culture and the numerous workers employed in relation to grape activities, the prevalence of allergy to pollen from *V. vinifera* remains unknown. Only a few studies have been published regarding general sensitization *V. vinifera* pollen exposure [5] and only two studies [6, 7] report *V. vinifera* pollen sensitization as an occupational allergy. Therefore, little is known about the relevance *V. vinifera* pollen in patients attending allergology units.

*V. vinifera* belongs to the Vitaceae family of plants, which is the only family of the order Vitales. Most plants of this family are distributed in tropical areas. Only species of the genus *Vitis* within this family were reported as an allergen, all of them as food allergens.





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*V. vinifera* pollinates from May to June, which coincides with other allergic 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 aim of this study was to investigate the prevalence of sensitization to *V. vinifera* pollen in La Rioja in the general population suffering from 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 in the study. Patients with other allergies (drug allergy) without respiratory symptoms were included as controls.

The study was approved by the Hospital San Pedro Ethics Committee (Logroño) (study number PI 530). All patients gave their oral consent to participate in the study. For patients aged under 18, a parent and/or the patient's legally authorized representative approved their taking part in the study.

### *Extract manufacturing*

*V. vinifera* pollen (Iberpolen, Jaen, Spain) was defatted with acetone and extracted with Phosphate buffered saline (PBS), according to internal manufacturing procedures (LETI Pharma S.L.u., Madrid, Spain). In short, vine pollen was subjected to two consecutive extraction processes for 4h and 18h at 4°C followed by centrifugation and collection of the supernatant. Finally, it was dialyzed, filtered, frozen and freeze dried. The protein





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content was measured by the Bradford method (Pierce Biotechnology, Rockford, IL, USA).

Non-standardized SPT 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

Skin prick tests (SPT) were performed on the volar side of the forearm for 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] using standardized lancets. A panel with different biologically standardized allergens (LETI Pharma) was used, including mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), moulds (*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 with fresh grapes was also performed. Histamine (10 mg/mL) and negative solutions were used as controls.

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

Serum samples were collected from positive patients who gave their consent. Subjects positive to vine pollen were asked about the area of residence and the years' clinical course of the respiratory symptoms.





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Protein profile (SDS–PAGE and 2D electrophoresis)

Protein profile of the extracts was investigated by SDS–PAGE. Briefly, ten micrograms of proteins from the *V. vinifera* extract were loaded in SDS–PAGE gels (2.67% C, 15% T acrylamide) under reducing conditions and stained with Oriole (Bio-Rad Laboratories, Hercules, CA, USA). Molecular weight (MW) of the different proteins were calculated with ImageQuantTL software version 8.1 (Cytiva, Uppsala, Sweden).

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

Specific IgE (CAP)

Serum specific IgE (sIgE) for each allergen with positive results by SPT was analysed in all patients by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden). In addition, 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 labelled using a biotin kit





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(Roche Diagnostics, Mannheim, Germany). Aliquots of 50 µL of *V. vinifera* biotin-labelled extract were incubated in streptavidin ImmunoCAPs (ThermoFisher Scientific) for 30 min and the assay continued as with the commercial ImmunoCAPs. The experiment was performed in the ImmunoCAP 100E system (ThermoFisher Scientific).

sIgE (ELISA)

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

Allergenic profile

The allergenic profile of patients was investigated by 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 (BioRad) and visualized by chemiluminescence. A serum pool was prepared with identical amounts from the seventeen individuals for whom bands in the immunoblot were recognized. This pool was used to identify the positive spots in a 2D immunoblot.





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Allergen sequencing

Spots recognized in the 2D immunoblot were excised from the gel, digested with trypsin, sequenced and identified by LC-MS/MS (Liquid Chromatography Mass Spectrometry/Mass Spectrometry) in the Proteomic Unit of Complutense University (Madrid, Spain).

Statistical analysis

Descriptive statistical analyses were used to investigate variables. Fisher's exact test was used for the comparison between *V. vinifera* positive and negative individuals. GraphPad Prism 9.1.0 software (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis.

**Results**

Patient Population

A total of 151 patients (89 females, 58.9%), with ages ranging from 4 to 76 years old (33.7±18.0 years) with respiratory symptoms and 30 controls (24 females, 80%) with ages ranging from 10 to 87 (49.8±19.6 years) were included in the study. The patients presented a previous history of rhinitis/rhinoconjuntivitis (50.9%), asthma (17.6%) or rhinitis and asthma (31.4%).

Skin prick test

One hundred and twenty-four patients had positive SPT to any of the allergenic sources tested. Twenty-seven patients (17.9%) with rhinitis and 30 controls without respiratory symptoms had negative SPT to all the allergens tested.

Sensitizations of positive patients are shown in Table 1. The most prevalent allergen was grass pollen with 66.9% of positive patients and the least prevalent was *A. alternata* with 5.6%. There was a positive association (statistical significance; $P<0.05$) between patients





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sensitized to *V. vinifera* and sensitization to other pollens, except for *P. radiata* pollen, and also with the molecular allergens profilin and LTP.

*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 seven were adolescents aged between 11 and 20 years.

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

They presented clinical course of respiratory symptoms of 5.7±4.0 years, varying between one year (four cases) and 16 years (one case). A total of 14, nine and 11 patients had clinical course of respiratory symptoms of less than three years, four to seven years and more than eight years, respectively.

Twenty-two cases had rhinitis (64.7%), 12 had bronchial asthma, 10 together with rhinoconjunctivitis (29.4%), seven of them from urban and five from semirural areas. Fourteen of the patients sensitized to *V. vinifera* lived in a semi-rural area (41.2%). This number decreased in the patients negative to pollen (21.4%) and was 35.4% in patients only positive to pollen. All data are shown in Table 2.

Eight patients (23.5%) were also sensitized to grape, none of them presented symptoms 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.





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Protein profile and 2D analysis

SDS–PAGE of the *V. vinifera* pollen extract is represented in Figure 4A. Several bands were observed in a MW 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 by SDS–PAGE. The distribution of the isoforms separated by the pI has a pH range between 3 and 8. The 15, 25 and 38 kDa bands presented at least four isoforms. Some bands were observed more clearly in 2D than in the SDS–PAGE, especially the 20 and 25 kDa bands (Figure 4B).

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, three 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 immunoblot (Figure 1).

A total of 20 individuals (60.6%) were positive to any of the three methods used to detect sIgE. Most (12; 60%) were positive with the three methods: ImmunoCAP, ELISA and immunoblot (Figure 2).

The MW and number of patients that recognized each band were: 14 kDa in 10 patients (30.3%), 21 and 36 kDa in six patients (18.2%), 27 and 63 kDa in five patients (15.2%), 55 kDa in four patients (12.1%), 43 kDa, 47 kDa bands and higher than 75 kDa bands appear in three patients (9.1%). This distribution can be observed in Figure 3.





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The pool of sera recognized spots in five areas of the 2D immunoblot (Figure 4C). Four of these spots contain various isoforms with different pI (isoelectric point). The spots recognized by the IgE had approximately 14, 21, 27, 38 and higher than 75 kDa.

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

Allergen identification

The identification of the five spots recognized by the IgE (Figure 4B) in the 2D immunoblot using LC-MS/MS analysis is shown in Table 3. These protein bands could be identified as allergens in *V. vinifera* and corresponds 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 beta-galactosidase (92.9 kDa). Profilin identity was confirmed by cross-reactivity with purified Pho d 2 (Figure S2, Supplementary material). Together with beta-galactosidase, spot 5 contains an uncharacterized protein. The allergenic prediction result was non-allergen (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 LC-MS/MS analysis (Figure 4B), this uncharacterized protein was ruled out as a vine allergen.

**Discussion**

The sensitization of patients to different pollen is influenced by the geographical area where they live. However, some pollens are not a common sensitizer, but could be a cause of allergy in areas with high exposure. This could be the situation of geographical areas near to a specific type of cultivation, such as wine, with large areas dedicated to the





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growth of *V. vinifera* and, therefore, with high exposure to its pollen. In this study, the prevalence of pollen from *V. vinifera* in a highly exposed area has been studied and the five most relevant allergens have been identified as being 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 respiratory allergic disease, a total of 34 patients out of 124 with pollen allergy (27.4%). This sensitization detected in the general population is higher than in other studies; 9% in the study by Feo *et al.* [5] and 14% was reported by Perontin *et al.* [7], in probable relation with a high exposure to this kind of culture in the region, suggesting that the general population can be sensitized without any type of occupational exposure that would favour said sensitization. According to these observations, in patients from our study sensitized to vine pollen, a higher percentage (64.7%) lives in urban areas, which supports the fact that further exposure is not required or there is no need to be in the professional setting for sensitization to this pollen. This also leads us to suppose that this pollen may be more aerovagant than what has been published up to now [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 explain more precisely or resolve this possibility.

All patients sensitized to *V. vitifera* pollen were also sensitized to other pollen, mainly grasses, *O. europea* and weeds (*P. lanceolata* and *Chenopodiaceae*). Cross-reactivity between *V. vinifera* and *P. pratense* and partial inhibition with other pollens, mainly *O. europea*, was observed by immunoblot inhibition. Cross reactivity between *V. vinifera* and other pollens was previously reported [5, 8]. Profilin was inhibited by other pollen extracts, and therefore, in patients exclusively sensitized to profilin, the positivity to *V. vinifera* could be due to cross-reactivity with other pollens. Against this backdrop, a





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sensitization sequence has been reported for grass pollen proteins, relating profilin to the evolution time of sensitized patients [11]. In our study, only seven patients were sensitized to profilin (nine by immunoblot), which suggests that most of our patients were sensitized earlier to vine pollen than to other pollens. In addition, the evolution time 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). This suggests a relationship with a primary sensitization to vine pollen in addition to that related to the evolution time. In a previous publication, sensitization to vine pollen and grape simultaneously was reported and revealed cross-reactivity between the allergenic structures of vine pollen and grapefruit [8]. In our study this was ruled out, as only eight patients (23.5%) of the *V. vinifera* pollen positive population were sensitized to grape and all of them presented good tolerance after its intake.

The limitation of this study remains in the determination of the clinical relevance of *V. vinifera* pollen sensitization. In all cases, sensitization to grasses or *O. europea* pollen was detected. The pollination period of these plants matches *V. vinifera* pollination, whose flowering occurs at the end of May or early June [10]. Additionally, the atmospheric concentration of *V. vinifera* pollen in the area is unknown; therefore, no relationship can be established between pollen levels and patient symptoms. Previous studies reported that in vineyard cultivation for workers with an occupational allergy, except in specific cases reported [10], symptoms were produced by grass pollen [7] and vine pollen did not have any clinical relevance [12]. However, it should not be ruled out that symptoms produced by vine pollen may be masked by coincidence with the symptoms produced by other more allergenic pollens and with a higher concentration [5, 10]. In these studies, clinical relevance was studied by means of bronchial and/or ocular provocation test, in addition to confirming the increase in respiratory symptoms in areas



 
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close to the cultivation, where there was more exposure to the pollen from this plant. This kind of analysis should be performed in our patients in regard to quantifying this relevance.

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

Allergens involved in *V. vinifera* sensitization were investigated in-depth. Profilin sensitization was observed in 27.2% of patients. In terms of the allergens identified profilin is a well-known panallergen with high cross-reactivity between plant allergens [13]. We also demonstrated this cross-reactivity by *in vitro* inhibition of *V. vinifera* profilin with Pho d 2.

However, another four 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 dehydrogenases family have been reported in moulds (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 allergen Tri a 34 and has a sequence identity of 84.6% compared to 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 [14,15,16]). The latter allergen has an identity of 79.5% with the vine allergen. Finally, beta-galactosidase was also recognized. This has been identified as an allergen in some Mediterranean trees such as olive or cypress. Its allergenic properties as well as its role in cross-reactivity has been reported [17]. Only





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one beta-galactosidase pollen is included in the Allergome database (Pho d 90 kD) corresponding to a palm tree allergen [18]. These four allergens could be considered specific to *V. vinifera* pollen.

In summary, in this study we have detected a significant sensitization to vine pollen in the general population in a vineyards area. Clinical significance is limited as all patients were also sensitized to other pollens with higher allergenicity that pollinize at the same time. Five novel vine allergens have been reported in a patient sensitized population. Further studies are needed to confirm their relationship with allergic symptoms, cross-reactivity with other pollen or foods and the clinical relevance of vine pollen allergy.

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**Conflicts of interest**

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

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

**Previous presentation**

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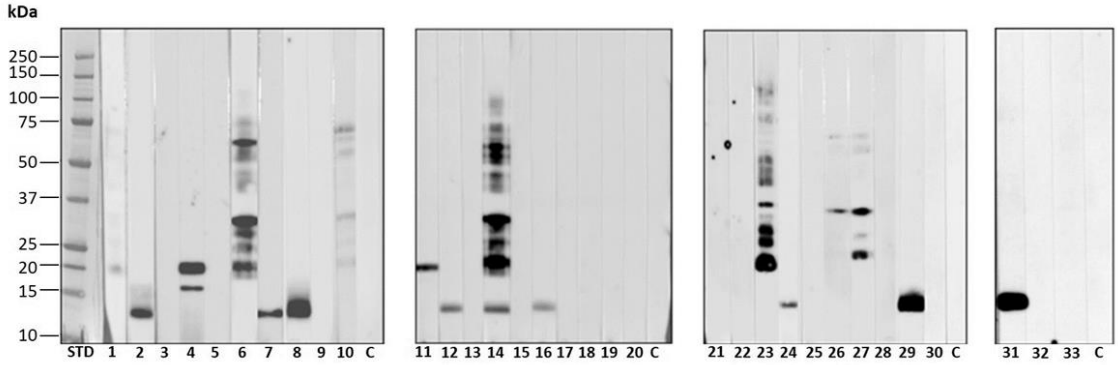
**Figure 1.** Allergenic profile. Immunoblot with individual sera from 33 patients positive to *V. vinifera* pollen by SPT. Twenty micrograms of protein from *V. vinifera* extracts were used in each lane; individual sera were diluted 1/5. C= the assay's negative control.








doi: 10.18176/jiaci.0849

**Figure 2.** Upset plots and Venn diagram representing the patients sIgE positive to *V. vinifera* by the different techniques used in the study.

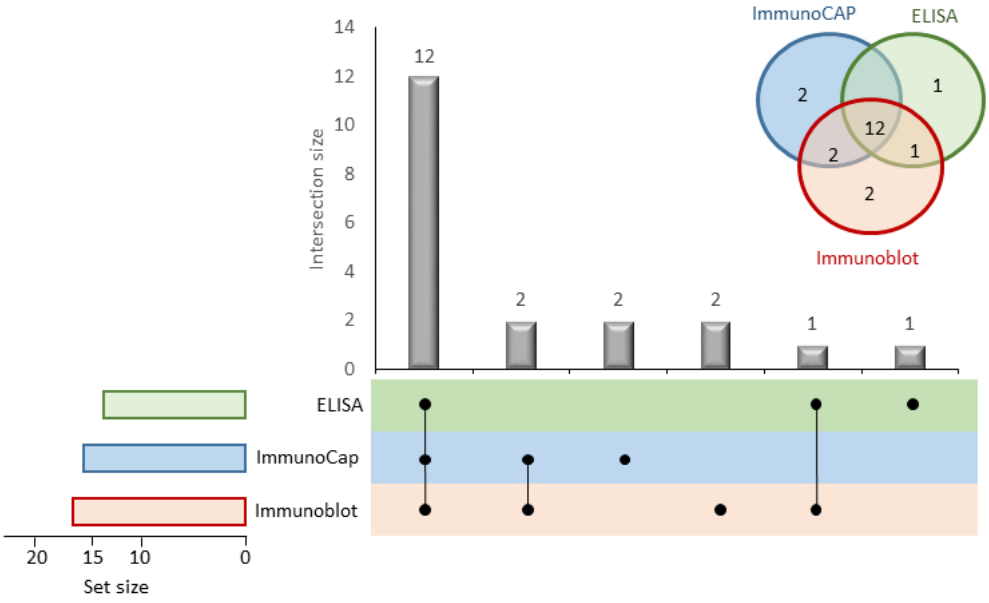


**Figure 3.** Allergogram. Percentage of patients recognizing each sIgE binding bands. Potential identification of the *V. vinifera* allergens are included in the graph.

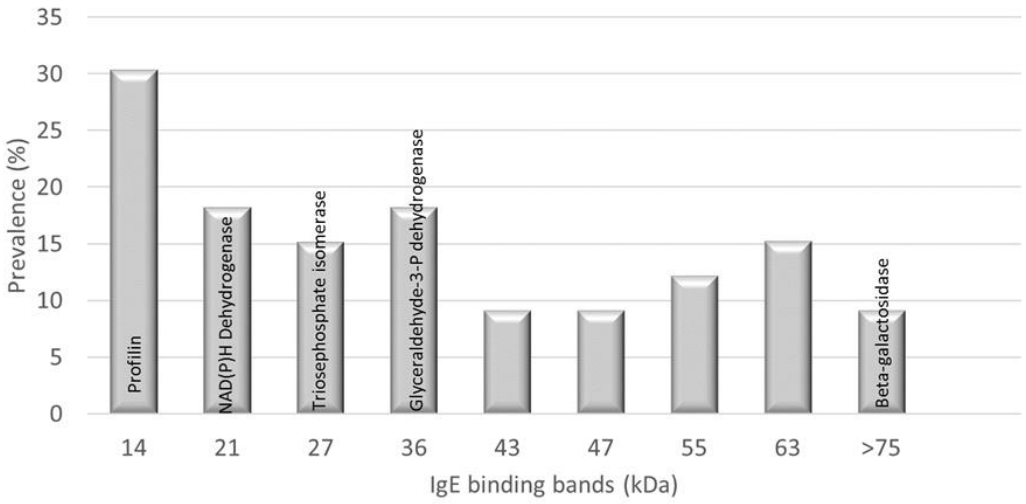


**Figure 4.** Protein and allergenic profile of *Vitis vinifera* pollen extract. A: SDS-PAGE of *V. vinífera* pollen extract (10 µg of protein). B: 2-D electrophoresis using a pH gradient from 3 to 10. Both gels (A and B) were stained with Oriole. C: 2D immunoblot showing the IgE binding of pooled sera to *V. vinifera* pollen extract. Spots chosen for protein identification by LC-MS/MS are marked in red in panel B.

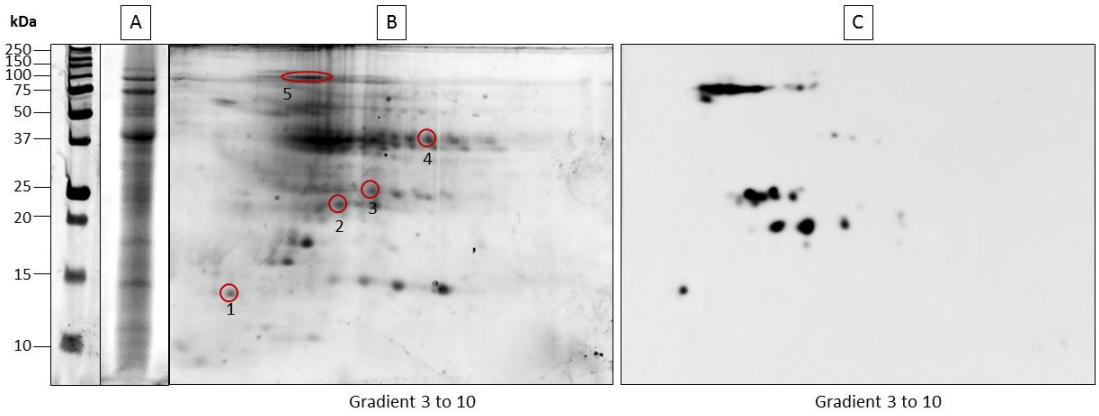


**Table 1.** Sensitization of the population.

| | **Total positive** | ***V. vinifera* negative** | ***V. vinifera* positive** | **Fisher's exact test (p value)** |
|---|---|---|---|---|
| n | 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%) | 0.0096 |
| *S. cereale* | 80 (64.5%) | 51 (56.6%) | 29 (85.3%) | 0.0031 |
| *A. vulgaris* | 18 (14.5%) | 8 (8.9%) | 10 (29.4%) | 0.0080 |
| *P. officinalis* | 13 (10.5%) | 5 (5.6%) | 8 (23.5%) | 0.0069 |
| *O. europea* | 57 (46.0%) | 28 (31.1%) | 29 (85.3%) | <0.0001 |
| *P. lanceolata* | 56 (45.2%) | 33 (36.6%) | 23 (67.6%) | 0.0025 |
| *B. alba* | 22 (17.7%) | 8 (8.9%) | 14 (41.5%) | <0.0001 |
| *C. avellana* | 11 (8.9%) | 3 (3.3%) | 8 (23.5%) | 0.0014 |
| *C. album* | 48 (38.7%) | 24 (26.7%) | 24 (70.6%) | <0.0001 |
| *S. kali* | 30 (24.2%) | 16 (17.8%) | 14 (41.5%) | 0.0097 |
| *F. excelsior* | 47 (37.9%) | 22 (24.4%) | 25 (73.5%) | <0.0001 |
| *Q. ilex* | 12 (9.7%) | 5 (5.6%) | 7 (20.6%) | 0.0182 |
| *P. acerifolia* | 31 (25.0%) | 15 (16.7%) | 16 (47.1%) | 0.0009 |
| *P. nigra* | 18 (14.5%) | 7 (7.8%) | 11 (32.4%) | 0.0012 |
| *C. arizonica* | 24 (19.4%) | 9 (10.0%) | 15 (44.1%) | <0.0001 |
| *P. radiata* | 2 (1.6%) | 1 (1.1%) | 1 (2.9%) | NS |
| **Molecular allergens** | | | | |
| Profilin | 13 (10.5%) | 6 (6.7%) | 7 (20.6%) | 0.0433 |
| LTP | 9 (7.3%) | 1 (1.1%) | 8 (23.5%) | 0.0001 |
| Grape | 9 (7.25%) | 1 (1.1%) | 8 (23.5%) | 0.0001 |

NS, non-significant.

**Table 2.** Demographic and clinical characteristics of the population.

| | Cases | | | | Controls |
|---|---|---|---|---|---|
| **SPT** | ***V. vinifera* +** | **Pollen -** | **Pollen +** | **Negative** | |
| N | 34 | 28 | 62 | 27 | 30 |
| Age (years±SD) | 29.6±16.8 | 33.2±19.4 | 32.2±13.8 | 43.6±23.1 | 49.8±19.6 |
| Sex, female (%) | 63.1 | 66.7 | 50.0 | 89.7 | 80 |
| Semirural residence (%) | 44.2 | 35.9 | 17.9 | 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 |
| Evolution (years±SD) | 5.7±4.0 | 7.3±7.2 | 8.1±5.8 | NA | NA |

Patients were selected by SPT. Negative column included patients that were negative to all the allergens tested.

**Table 3.** *V. vinifera* pollen allergens identified by LC-MS/MS. Peptides identified in the assay are marked in bold in the corresponding amino acid sequence.

| Spot | Accession Number | Protein name | % Coverage | Peptides matches | Theoretical MW (kDa) |
|---|---|---|---|---|---|
| **1** | A5ASF9 and A5BLM8 | Profilin | 66 | 9 | 14.2 |
| MSWQTYVDDHLMCEIDGQGQHLTAAAIVGHDGSVWAQSTSFPEFK**TPEITGIMNDFAEPGHLAPTGLYL GGTKYMVIQGEPGAVIRGKKGSGGITIKKTGQALVFGIYEEPVTPGQCNMVVERLGDYLVDQGL** | | | | | |
| **2** | A5AS18 | NAD(P)H dehydrogenase | 58 | 13 | 21.7 |
| MVTKVYIVYYSMYGHVEK**LAEEIKKGAASVEGVEAKLWQVPETLPEEVLGK**MSAPPK**SDTPIITPTDLA EADGFVFGFPTRFGMMAAQFKAFLDATGGLWR**TQQLAGKPAGIFYSTGSQGGGQETTALTAITQLVHHG MIFVPIGYTFGAGMFEMEK**VKGGSPYGAGTFAGDGSRQPSELELEQAFHQGKYIAGITKK**LKEAA | | | | | |
| **3** | D7TLU7 | Triosephosphate isomerase | 74 | 14 | 27.1 |
| MGRK**FFVGGNWK**CNGTGEEVKK**IVSTLNAGEVPSGDVVEVVVSPPFVFLPLVKSTLRPDFHVAAQNCWV K**KGGAFTGEISAEMLVNLGIPWVIIGHSER**RLLLNESNEFVGEKVAYALSKGLKVIACVGETLEQRESG STMEVVAAQTKAIADKVSNWANVVLAYEPVWAIGTGKVATPAQAQEVHSELR**NWFQANASPEVAATIR**I IYGGSVSGANCKELAAKPDVDGFLVGGASLKPEFIDIIK**SAEVKKNC | | | | | |
| **4** | A0A438H737 | Glyceraldehyde-3-phosphate dehydrogenase | 74 | 28 | 36.5 |
| MAKIKIGINGFGRIGRLVARVALQRDDVELVAVNDPFINTDYMTYMFK**YDSVHGQWK**HHDIK**VKDSKTL LFGDKAVTVFGAKNPEEIPWGEAGAEYVVESTGVFTDKDK**AALHLKGGAKK**VIISAPSSNAPMFVVGVN EKEYKSNIDIVSNASCTTNCLAPLAKVIHDKFGIVEGLMTTVHSITATQKTVDGPSMKDWR**GGR**AASFN IIPSSTGAAKAVGK**VLPALNGK**LTGMAFRVPTADVSVVDLTVRTEKKASYDDIKAAIKAESEGNLKGIL GYTEDEVVSSDFLGDSRSSIFDAKAGIALNENFIKLVSWYDNEWGYSSR**VVDLIRHIDSTK | | | | | |
| **5** | D7TCB5 | Beta-galactosidase | 31 | 27 | 92.9 |
| MYRTYFLGNTSVASSKNATHAISFCVLFVLLNVLASAVEVSYDGRALIIDGKR**RVLQSGSIHYPRSTPE MWPDLIR**KAKAGGLDAIETYVFWNVHEPLRR**EYDFSGNLDLIRFIQTIQAEGLYAVLR**IGPYVCAEWTY GGFPMWLHNMPGIEFRTANKVFMNEMQNFTTLIVDMAKQEK**LFASQGGPIIIAQIENEYGNIMAPYGDA GK**VYVDWCAAMANSLDIGVPWIMCQQSDAPQPMINTCNGWYCDSFTPNNPNSPK**MWTENWTGWFK**NWGG KDPHR**TAEDLSYSVAR**FFQTGGTFQNYYMYHGGTNFGR**VAGGPYITTSYDYDAPLDEFGNLNQPK**WGHL KDLHTVLKSMEETLTEGNITTIDMGNSVEVTVYATQKVSSCFFSNSNTTNDATFTYGGTEYTVPAWSVS ILPDCKKEVYNTAK**VNAQTSVMVKNKNEAEDQPASLKWSWRPEMIDDTAVLGK**GQVSANRLIDQKTTND RSDYLWYMNSVDLSEDDLVWTDNMTLRVNATGHILHAYVNGEYLGSQWATNGIFNYVFEEKVKLKPGKN LIALLSATIGFQNYGAFYDLVQSGISGPVEIVGR**KGDETIIKDLSSHK**WSYKVGMHGMAMK**LYDPESPY K**WEEGNVPLNRNLTWYKT**TFKAPLGTDAVVVDLQGLGK**GEAWVNGQSLGRYWPSSIAEDGCNATCDYRG PYTNTKCVRNCGNPTQRWYHVPRSFLTADENTLVLFEEFGGNPSLVNFQTVTIGTACGNAYENNVLELA CQNRPISDIKFASFGDPQGSCGSFSK**GSCEGNKDALDIIKKACVGKESCSLDVSEKAFGSTSCGSIPKR** LAVEAVC | | | | | |