

ORIGINAL ARTICLE

Rhinitis, sinusitis, and upper airway disease

Profilin is a marker of severity in allergic respiratory diseases

Javier Ruiz-Hornillos^{1,2,3} | M. Angeles López-Matas⁴ | Pilar Berges Jimeno⁵ |
 Aythamy Henríquez^{1,2,3} | Sandra Blanco^{1,2,3} | Marta Seoane-Rodríguez^{1,2,3} |
 Ignacio Mahillo^{6,7} | Jerónimo Carnés⁴ 

¹Allergy Unit, Hospital Universitario Infanta Elena, Valdemoro, Madrid, Spain

²Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain

³Health Research Institute-Fundación Jiménez Díaz University Hospital (IIS-FJD), Madrid, Spain

⁴R&D Allergy & Immunology Unit, Laboratorios LETI S.L.u, Madrid, Spain

⁵Allergology Service, Hospital Universitario Ramón y Cajal, Madrid, Madrid, Spain

⁶Epidemiology, Fundación Jiménez Díaz, Madrid, Spain

⁷Department of Medicine, Universidad Autónoma de Madrid, CIBERES, Instituto Carlos III, Madrid, Spain

Correspondence

Jerónimo Carnés, PhD, Research & Development, Laboratorios LETI, S.L.U., Calle del Sol n 5, 28760 Tres Cantos. Email: jcarnes@leti.com

Funding information

Supported by a research grant of the SEAIC Foundation.

Abstract

Background: The capacity of profilin to induce allergic symptoms in patients with respiratory allergy has been questioned. In this sense, the aim of this study was to investigate the correlation between profilin exposure and induction of symptoms in a prospective case-control study.

Methods: The concentration of profilin as well as pollen levels in the air was measured. A diary score of symptoms was collected from allergic patients. Seventy-nine individuals were included in the study; fifty cases and 28 controls were positive or negative to profilin, respectively. Conjunctival and bronchial provocation tests were performed with purified profilin (Pho d 2) in a subgroup of cases and controls.

Results: Profilin was detected in the environment on 133 days (maximum peak of 0.56 ng/m³). A positive correlation between profilin and pollen count of *Olea* and *Poaceae* was observed ($p = 0.24$; $P < .001$). Intensity of total, nasal and ocular symptoms was statistically higher in cases than in controls ($P < .001$). The risk of suffering symptoms, measured by the percentage of patients who presented any of the symptoms each day, was also higher in cases than in controls. The provocation test was positive in 95% of bronchial and 90% of conjunctival challenges in cases, and negative in all controls.

Conclusions: Profilin was detected in the environment and had the ability to induce a specific allergen response. Patients sensitized to this panallergen showed more symptoms and were more likely to have symptoms. Therefore, sensitization to profilin seems to be a marker of severity in patients with rhinoconjunctivitis and asthma mediated by pollen.

KEYWORDS

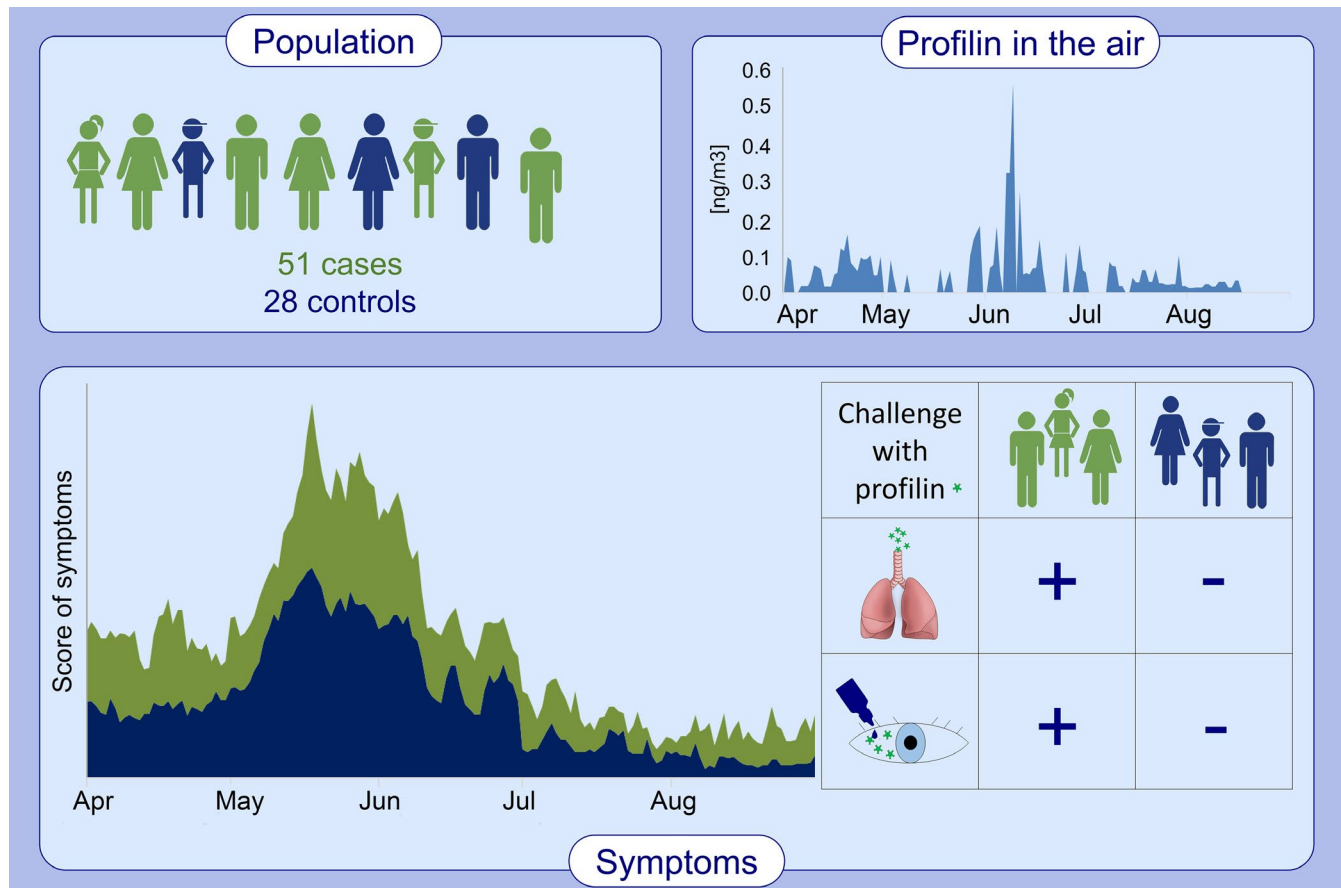
aeroallergen, allergen quantification, profilin, provocation test, respiratory symptoms

1 | INTRODUCTION

Profilin is a ubiquitous family of proteins of about 12–16 kDa present in eukaryotic cells and is involved in the control of actin polymerization.^{1,2} They have been reported as panallergens, and their similar tertiary structure, even among taxonomically separated plant species, is the cause of their high cross-reactivity.^{3,4}

The sensitization profile of patients is highly variable (20%–30% of patients with pollen allergy) and mainly depends on geographical distribution

and other concomitant and predominant allergens.^{5,6} In Central and Northern Europe, profilin sensitization has mainly been associated with respiratory allergy to birch,⁷ while in Southern Europe, it is associated with exposure to grasses, which sensitize up to 60% of patients.⁸ On the contrary, in some areas of Australia, where ragweed pollen is predominant, 50% of patients allergic to pollen are sensitized to profilin.³ Some authors attribute these percentages to co-recognition, or cross-reactivity.^{4,9,10} On the contrary, some have recently been questioning the lack of relevance of profilin recognized so far,^{11,12} revealing that early sensitization



GRAPHICAL ABSTRACT

Profilin is detected in the environment and has the capacity to induce a specific allergen response as evidenced by bronchial and conjunctival challenges. Patients sensitized to profilin showed more and severe allergic symptoms and had more probability to develop allergic symptoms than the allergic control population negative to profilin. Sensitization to profilin seems to be a marker of severity in pollen allergic patients with rhinoconjunctivitis and asthma.

to profilin could be a marker of predisposition to more severe allergic disease.¹³ The presence of profilin-specific IgE has been associated with an increased risk of sensitization to multiple pollens and the presence of food allergy,¹⁴ and higher risk of allergic reactions to specific immunotherapy.¹⁵

With the aim of investigating the capacity of profilin to induce allergic symptoms, the objective of this study was to establish a correlation between clinical symptoms and profilin exposure by challenging a pollen allergic population to a conjunctival and bronchial provocation test with purified profilin, in a prospective case-control study. Additionally, correlation between the concentration of profilin in the environment and severity of symptoms was also investigated.

2 | MATERIALS AND METHODS

2.1 | Patient population

This study consists of a case-control study, with a sample population of patients older than 14 years who came to the Hospital Universitario Infanta Elena (Valdemoro, Madrid, Spain) for the first time, due to allergic respiratory pathology. Clinical cases included patients with

rhinitis, rhinoconjunctivitis and/or asthma and a positive skin prick test (SPT) to profilin, while the control group included patients with rhinitis, rhinoconjunctivitis and/or asthma but negative to profilin. Serum samples from cases and controls were collected for further studies.

All patients gave written consent to participate in the study (or parents/legal representative in the case of patients between 14 and 18 years of age). Individuals with severe atopic dermatitis, uncontrolled bronchial asthma or any other severe respiratory pathology that limits performing diagnostic tests and evaluation of the results thereof, or those who declined consent, were excluded from the study.

The study protocol was approved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz (Madrid, Spain) (Number EO172011FJD).

2.2 | Symptom Diary Cards

Throughout the year, all the patients completed diary cards on which they recorded their conjunctival, nasal and bronchial symptom scores according to the following scale^{16,17}: 0, no symptoms; 1, mild symptoms

(slight nasal obstruction, slightly runny nose, or occasional sneezing or itching of the eyes); 2, moderate symptoms (moderate nasal obstruction, moderately runny nose, some sneezing and congestion, some ocular itching or mild asthma); and 3, severe symptoms (complete nasal obstruction, almost continuously runny nose, frequent sneezing or ocular symptoms or asthma attacks) (Figure S1).

2.3 | In vivo studies

2.3.1 | Skin prick tests

All individuals recruited were skin prick tested with a standard battery of biologically standardized aeroallergens including a mixture of grasses, *Lolium perenne*, *Secale cereale*, *Cynodon dactylon*, *Olea europaea*, *Cupressus arizonica*, *Platanus hybrida*, *Parietaria judaica* and *Salsola kali*, moulds (*Alternaria alternata*), mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal epithelia (cat and dog), and with purified profilin from *Phoenix dactylifera* (Pho d 2, 50 µg/mL¹⁸) (Laboratorios LETI SLu, Madrid, Spain).

2.3.2 | Conjunctival allergen challenge

Conjunctival challenges were performed according to the usual technique¹⁹ with purified Pho d 2 in 5 concentrations increasing from 0.003 µg/ml to 3 µg/ml dissolved in saline solution (0.85% NaCl, phosphate buffer 7 mM), following the recently recommended evaluation criteria.²⁰ Conjunctival challenges were performed in 10 cases and 5 controls outside the symptom registration period so as not to interfere with the symptom diary card.

2.3.3 | Bronchial challenge

Specific bronchial challenge tests were performed with purified profilin¹⁸ in serial dilutions (from 0.15 µg/ml to 30 µg/ml) in sterile PBS (phosphate-buffered saline) by the tidal flow method doubling concentrations of the antigen dissolved in sterile PBS from 0.15 µg/ml to 30 µg/ml, as previously reported.^{21,22} Late response with peak flow was controlled in the 24 hours after the test. Specific bronchial challenge tests were performed in 20 cases and 10 controls outside the symptom registration period for diary cards.

2.4 | In vitro studies

2.4.1 | sIgE and immunoblotting

Specific IgE (sIgE) to rPhl p 12 and rBet v 2 was measured by ImmunoCap (Thermo Fisher, Uppsala, Sweden). In addition, recognition of Pho d 2 by the patients' sera was analysed by immunoblot as reported.¹⁸

2.5 | Aerobiological studies

2.5.1 | Air sampling

A volumetric air sampler (Air Sentinel, Quan-Tec-Air Inc, Rochester, Minnesota, USA) adapted for outdoor use²³ was used for aeroallergen collection. The sampler was placed 8.26 m above street level in Valdemoro (coordinates 40°11'53"N 3°41'50"O) and run continuously throughout 2012 with an air flow of 10 m³/h. Airborne particles were collected onto polytetrafluoroethylene membranes (Merck Millipore). Sampling time for each filter was 24 hours, which represents 240 m³ of air per sample, and filters were replaced at approximately the same time each day. After removal, filters were sealed in plastic bags and stored at 4°C until extraction.

2.5.2 | Filter extraction and allergen quantification

The upper layer of 220 filters was separated and individually placed in tubes containing 2 ml of 0.01 M PBS. Tubes were stirred until the filter was completely soaked and left for overnight extraction in a rotary mixer at 4°C. Afterwards, the liquid content was collected and the filter discarded.

Allergen content was measured by ELISA inhibition.¹⁶ In short, purified Pho d 2 was used as standard (from 1.95 ng to 1000 ng). Microplates were coated with profilin at 1 µg/well. Samples were incubated with polyclonal anti-Pho d 2 antibodies produced in rabbit as previously reported¹⁶ (dilution 1:30 000). Allergen concentrations were estimated using a standard curve and were based on inhibition capacity; final results were expressed in ng/m³ of air.

2.5.3 | Pollen count

Aerobiological sampling was performed from 1st January to 31st December 2012 with a Burkard pollen collector placed in Valdemoro one metre from the volumetric air sampler. Samples were examined under optic microscope with a 100× objective lens. Pollen concentrations were expressed as pollen grains/m³ of air.

2.6 | Statistical analysis

Fisher's exact test was used to study the relationship between study variables. The Mann-Whitney rank sum test was used to compare numerical numbers obtained for the different groups. Linear regression and logistic regression models were used to evaluate the relationship of variables to the intensity and presence of symptoms, respectively. Scatter plots were used, and the Spearman correlation coefficient was calculated to evaluate IgE concentration and symptom severity. The software GraphPad Prism 7 was used for analyses.

3 | RESULTS

3.1 | Patient population

Seventy-nine patients (mean age 30.1 ± 8.5 years) were included in the study: 51 positive to profilin (cases) and 28 negative to profilin (controls) based on SPT to Pho d 2. The characteristics of the population are shown in Table 1.

All the population had rhinitis and conjunctivitis, whereas 38 cases (74.5%) and 22 controls (78.6%) also had asthma. Statistically significant differences were found in the years of evolution of asthma, with more years in the cases than in the controls (5.8 ± 4.9 years vs 2.9 ± 2.5 years; $P < .05$).

3.2 | In vivo studies (profilin diagnosis)

3.2.1 | SPT

Mean value for wheal sizes induced by profilin in the 51 cases was 38.4 ± 23.7 mm² (individual wheal sizes are shown in Figure S2).

All patients were sensitized to pollen; Forty-four cases (86.3%) and 13 controls (46.4%) were sensitized to three or more pollens ($P < .001$) which indicates that patients sensitized to profilin were sensitized to more species than patients not sensitized to profilin. Most profilin-sensitized patients were sensitized to *Cynodon dactylon* (92.2%), with statistically significant differences in comparison with profilin-negative patients (53.6%) ($P < .001$). There was also a significant correlation between sensitization to profilin and to *Platanus acerifolia* (76.5% vs. 17.9%) ($P < .001$) and *Parietaria judaica* (21.6% vs. 3.6%) ($P < .05$) (Table 1).

3.2.2 | Conjunctival challenges

Conjunctival challenges were performed in 15 patients, who gave their consent for the test 10 cases and 5 controls, being positive in 9 of the 10 cases. The median concentration that induced the reaction was 0.3 µg/ml (Table 2 and Table S1); all 5 controls had a negative challenge test.

3.2.3 | Bronchial challenges

Among patients diagnosed with asthma, bronchial challenge with purified Pho d 2 was performed on the first 20 cases and 10 controls who gave their consent for the test (Table 3). Nineteen cases (95%) had a positive bronchial challenge with profilin, with amounts ranging from 0.31 to 20 µg/ml, the mean PC20 (provocative concentration causing a 20% fall in Forced Expiratory Volume in the first second [FEV1]) being 10.55 ± 11.87 µg/ml. A statistically significant difference was observed ($P < .001$), both in the final FEV1 and in the percentage FEV1 decrease from baseline

when comparing cases and controls. FEV1 decreased a mean of 24.3% for cases and 5.9% for controls. A total of 60% of cases presented additional symptoms during the test, the most common being nasal symptoms (35%), cough (20%) and palatal pruritus (20%) (Table S2). There was no late response in peak flow records during the 24 hours after the test.

All controls had negative bronchial challenge with purified profilin.

3.3 | In vitro studies (slgE and immunoblot)

slgE to rPhl p 12 was positive in 38 cases (74.5%), with values of 4.8 ± 10 kU/l and rBet v 2 was positive in 42 cases (82.3%) with values of 6.2 ± 10.8 kU/l (Figure S2). Both profilins were negative in controls.

Forty-two of the 51 cases (82.4%) recognized nPho d 2 by immunoblot (Figure S2). Nine sera that did not recognize nPho d 2 were also slgE negative to rPhl p 12 and rBet v 2. None of the controls recognized profilin in the immunoblots (data not shown).

3.4 | Aerobiological and clinical studies

3.4.1 | Profilin quantification on filters and correlation with the pollen count of different species

A total of 220 filters were analysed. The maximum value was obtained on 10th June with 133.4 ng of profilin in the filter (0.56 ng/m³ of air). The distribution of profilin during the year is shown in Figure 2. Profilin was detected in the environment in 133 days (36.5% of the year). For 58 days (15.9% of the year), profilin concentrations higher than 10 ng were observed in the filters (>0.04 ng/m³ of air). The month with the highest profilin content was June, but there were also other smaller peaks in April and the end of July (Figure 1).

The profilin peak appeared some days after the pollen peak of *Olea* and *Poaceae* (Figure 1A, 1B). Fifty different types of pollen were identified during the period of study. Plants with pollen counts during the whole year higher than 1000 pollen grains/m³ of air were as follows: *Cupressaceae* (2195 pollen grains/m³), *Olea* (2917), *Pinaceae* (1994), *Platanus* (1072), *Poaceae* (2504) and *Quercus* (12 747). Among them, profilin has been identified as an allergen in *Olea*, *Platanus* and *Poaceae*. The correlation coefficient was calculated for different pollens, with respect to profilin levels. *Olea* and *Poaceae* pollens showed a positive correlation ($\rho = 0.24$; $P < .001$). In the case of *Platanus* ($\rho = 0.41$; $P < .001$), the peak of pollination was two months before the peak of profilin (Figure 1C). Pollen levels of *Quercus* were significantly higher than other species and pollinated approximately one month before the highest peaks of profilin ($\rho = 0.36$; $P < .001$) (Figure 1E). Finally, in the case of *Plantago* ($\rho = 0.39$; $P < .001$), the level of pollen grains/m³ was low (Figure 1D).

TABLE 1 Description of the study population

	Total	Cases Profilin positive	Controls Profilin negative	p value
n	79	51 (64.6%)	28 (35.4%)	
Age (years $\pm$ SD)	30.1 $\pm$ 8.5	29.3 $\pm$ 8.5	31.5 $\pm$ 8.7	NS
Female n (%)	49 (62.0%)	33 (64.7%)	16 (57.1%)	NS
Respiratory symptoms				
Rhinitis	79 (100%)	51 (100%)	28 (100%)	NS
Years of evolution for rhinitis	8.6 $\pm$ 5.1	9.2 $\pm$ 4.9	6.9 $\pm$ 5.4	NS
Conjunctivitis	79 (100%)	51 (100%)	28 (100%)	NS
Asthma	60 (75.9%)	38 (74.5%)	22 (78.6%)	NS
Years of evolution for asthma	4.7 $\pm$ 4.4	5.8 $\pm$ 4.9	2.9 $\pm$ 2.5	$P < .05$
Sensitization to aeroallergens (SPT)				
Mites	24 (30.4%)	15 (29.4%)	9 (32.1%)	NS
Moulds	18 (22.8%)	12 (23.5%)	6 (21.4%)	NS
Epithelia	47 (59.5%)	31 (60.8%)	16 (57.1%)	NS
Pollen (All)	79 (100%)	51 (100%)	28 (100%)	NS
Grasses	73 (92.4%)	47 (92.2%)	26 (92.9%)	NS
<i>Lolium perenne</i>	74 (93.7%)	48 (94.1%)	26 (92.9%)	NS
<i>Secale cereale</i>	75 (94.9%)	49 (96.1%)	26 (92.9%)	NS
<i>Cynodon dactylon</i>	62 (78.5%)	47 (92.2%)	15 (53.6%)	$P < .001$
<i>Olea europea</i>	67 (84.8%)	43 (84.3%)	24 (85.7%)	NS
<i>Cupressus arizonica</i>	44 (55.7%)	30 (58.8%)	14 (50.0%)	NS
<i>Platanus acerifolia</i>	44 (55.7%)	39 (76.5%)	5 (17.9%)	$P < .001$
<i>Parietaria judaica</i>	12 (15.2%)	11 (21.6%)	1 (3.6%)	$P < .05$
<i>Salsola kali</i>	36 (45.6%)	27 (52.9%)	9 (32.1%)	NS
Sensitization to 3 or more pollens	50 (73.4%)	44 (86.3%)	13 (46.4%)	$P < .001$

Abbreviations: NS, non-significant.

Percentages calculated from the "n" in each group.

TABLE 2 Conjunctival challenge to profilin

0	Result	Final concentration
Case 13	Pos	3
Case 15	Pos	0.3
Case 20	Pos	0.03
Case 27	Pos	1.5
Case 31	Neg	3*
Case 33	Pos	0.3
Case 34	Pos	0.3
Case 38	Pos	0.3
Case 39	Pos	0.3
Case 46	Pos	1.5
Control 6	Neg	3*
Control 7	Neg	3*
Control 8	Neg	3*
Control 13	Neg	3*
Control 25	Neg	3*

Abbreviations: Neg, Negative result; Pos, Positive result.

*No induction of positive response.

3.4.2 | Patient symptoms diary

Total (Figure 2A), bronchial (Figure 2B), nasal (Figure 2C) and conjunctival (Figure 2D) symptoms were compared between cases and controls.

It was observed that mean intensity of total symptoms, throughout the year, was on average 0.56 points higher in cases than in controls, 95% CI (0.43,0.70) $P < .001$ (Figure 2A); nasal symptoms were 0.26 points higher in cases than in controls, CI (0.18,0.34) $P < .001$ (Figure 2C); and conjunctival symptoms 0.27 points higher in cases than in controls, CI (0.23,0.31) $P < .001$ (Figure 2D).

For bronchial symptoms, statistically significant differences ($P = .020$) between cases and controls were limited to the presence of profilin (from March to August) in the environment (0.10 points; CI [0.02, 0.19]) (Figure 2B).

The risk of suffering asthma was higher in cases than in controls with OR 1.32 95% CI (1.19, 1.46) ($P < .001$). The same occurred with nasal and conjunctival symptoms, with an OR of 1.15 95% CI (1.08, 1.23) ($P < .001$) and OR of 1.62 95% CI (1.47, 1.79) ($P < .001$), respectively.

No correlation was detected between the intensity of these symptoms and sIgE levels to rPhl p 12 and rBet v 2.

TABLE 3 Bronchial challenge to profilin

Cases	FEV1 baseline (L)	FEV1 post-saline (L)	FEV1 Final (L)	FEV1 decrease (%) from baseline	Last concentration of profilin ($\mu\text{g/ml}$)	PC20 ($\mu\text{g/ml}$)	Result
Case 2	3.38	3.51	2.62	25.4	5.00	7.48	Pos
Case 3	1.87	1.78	1.27	28.7	5.00	6.33	Pos
Case 12	3.00	3.01	2.35	21.0	10.00	18.67	Pos
Case 13	3.14	3.02	2.17	28.1	5.00	7.32	Pos
Case 15	2.62	2.59	2.44	5.8	20.00	N/A	Neg
Case 16	2.64	2.31	1.67	27.7	2.50	3.24	Pos
Case 17	2.35	2.34	1.84	21.4	0.31	0.43	Pos
Case 21	2.86	2.9	2.09	28.0	10.00	12.86	Pos
Case 22	2.94	2.99	2.26	24.4	1.21	1.46	Pos
Case 24	3.14	3.19	2.55	20.1	20.00	39.36	Pos
Case 26	4.25	4.33	3.46	20.0	10.00	19.77	Pos
Case 27	3.02	3.07	2.16	29.7	20.00	26.96	Pos
Case 31	4.15	4.07	3.13	23.1	2.50	2.98	Pos
Case 32	3.35	3.36	2.44	27.4	2.50	3.45	Pos
Case 33	3.17	3.02	1.94	35.8	1.25	1.27	Pos
Case 34	2.38	2.27	1.75	26.5	2.50	3.96	Pos
Case 35	4.14	4.11	3.22	21.7	20.00	34.99	Pos
Case 38	2.61	2.55	2.00	21.6	1.25	2.13	Pos
Case 48	3.25	3.21	2.44	24.0	1.25	1.19	Pos
Case 51	3.96	3.93	2.94	25.2	5.00	6.65	Pos
Mean (SD)	3.11 (0.64)	3.08 (0.68)	2.34 (0.55)	24.3 (5.9)	Median: 5.00 $\mu\text{g/ml}$	10.55 (11.87)	
Controls	FEV1 baseline (L)	FEV1 post-saline (L)	FEV1 Final (L)	FEV1 decrease (%) from baseline	Last Concentration of profilin ($\mu\text{g/ml}$)	Total accumulated dose	Result
Control 03	3.12	3.04	2.87	5.6	20	39.83	Neg
Control 04	3.66	3.70	3.43	7.3	20	39.83	Neg
Control 08	3.88	3.71	3.71	0.0	20	39.83	Neg
Control 11	2.69	2.79	2.80	0.3	20	39.83	Neg
Control 12	3.46	3.39	3.27	5.4	20	39.83	Neg
Control 13	4.07	4.11	3.83	7.8	20	39.83	Neg
Control 15	2.59	2.51	2.50	0.4	20	39.83	Neg
Control 23	3.78	3.80	3.32	12.2	20	39.83	Neg
Control 25	2.78	2.52	2.34	7.2	20	39.83	Neg
Control 28	4.25	4.11	3.92	4.4	20	39.83	Neg
Mean (SD)	3.43 (0.6)	3.37 (0.61)	3.20 (0.55)	5.1 (3.9)			

Abbreviations: FEV1, Volume exhaled at the end of the first second of forced expiration; Neg, Negative result; N/A, Not Applicable; PC20, provocative concentration causing a 20% fall in FEV1; Pos, Positive result.

4 | DISCUSSION

Identification of allergens in the environment has become important for the control of allergic diseases and for establishing a relationship with clinical symptoms.^{16,24,25} In the present study, we quantified the profilin levels in the environment and investigated their correlation with the clinical symptoms of pollen allergic patients. In addition, the capacity of profilin to induce allergic symptoms was demonstrated. To our

knowledge, this is the first time that the capacity of profilin to induce clinical allergic symptoms has been demonstrated in patients in real life.

We set up a method for quantifying profilin in the air over the course of a year, which resulted useful for establishing a correlation with the clinical symptoms of our population. Although the methodology for extraction and quantification of profilin in the filters rendered good results, more sophisticated methods that obtain a more accurate concentration would provide more exact data.

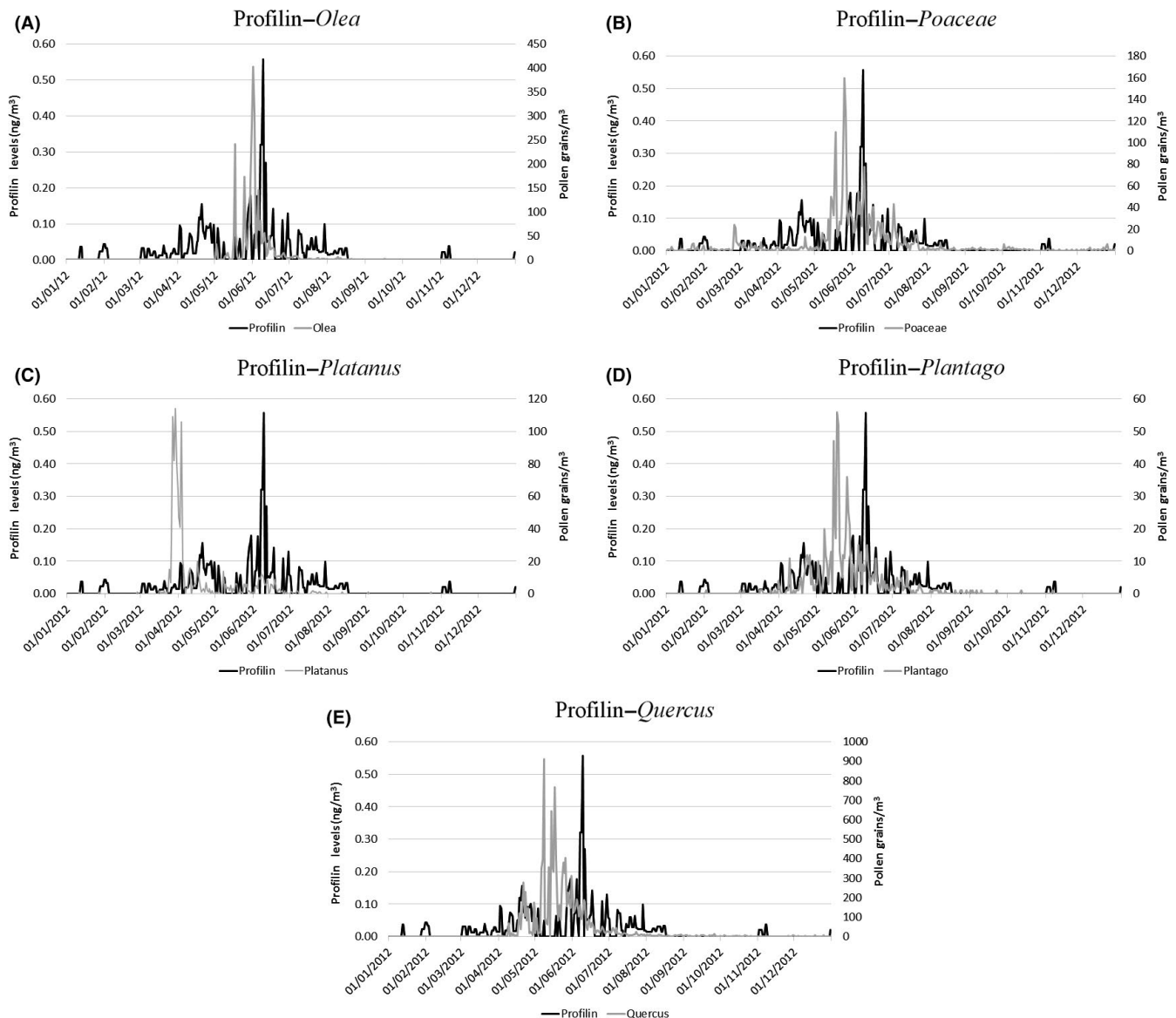


FIGURE 1 Pollen counts and profilin levels during the study period. (A) *Olea*; (B) *Poaceae*; (C) *Platanus*; (D) *Plantago* and (E) *Quercus*. The profilin peak appeared after the maximum pollen peak of *Olea* and *Poaceae*

Regarding the concentration of profilin in the environment, June showed the highest concentration followed by May and April, corresponding to the highest peaks of pollen grains in the air (especially from grasses and olive trees), although a lag of a few days was observed. We hypothesize that profilin requires certain meteorological conditions to be detected and released from pollen grains. Another finding we observed was that the presence of profilin was not limited to the spring season, with different concentrations detected out of this period. This fact may be related to the different concentration of profilin in different pollen species. In this sense, *Lolium perenne* is one of the pollens with a higher percentage of relative profilin content,⁸ while the lower percentage in olive trees or *Plantago* suggests a lower contribution.⁸ *Quercus* may contribute significantly in correlation with the high levels of pollen grains, although the contribution of this tree to

allergic symptoms is almost irrelevant at Madrid's latitude. This is especially important because the implication of profilin for clinical symptoms might vary despite the high cross-reactivity.²⁶ In general, the clinical implications of small amounts of profilin remain unknown, although different studies have suggested that profilin may enhance allergic inflammation.²⁷

A positive correlation between pollination and clinical allergic symptoms is well established. However, although the presence of allergens in the atmosphere (such as profilin) and their implication in clinical symptoms can be extrapolated, it is necessary to establish its allergenic relevance and capacity to induce allergic symptoms. In this way, our study confirms the capacity of profilin to produce an allergen-specific response by using bronchial and conjunctival provocation tests. These results confirm that profilin should be considered as a respiratory allergen. These observations are in agreement with previous

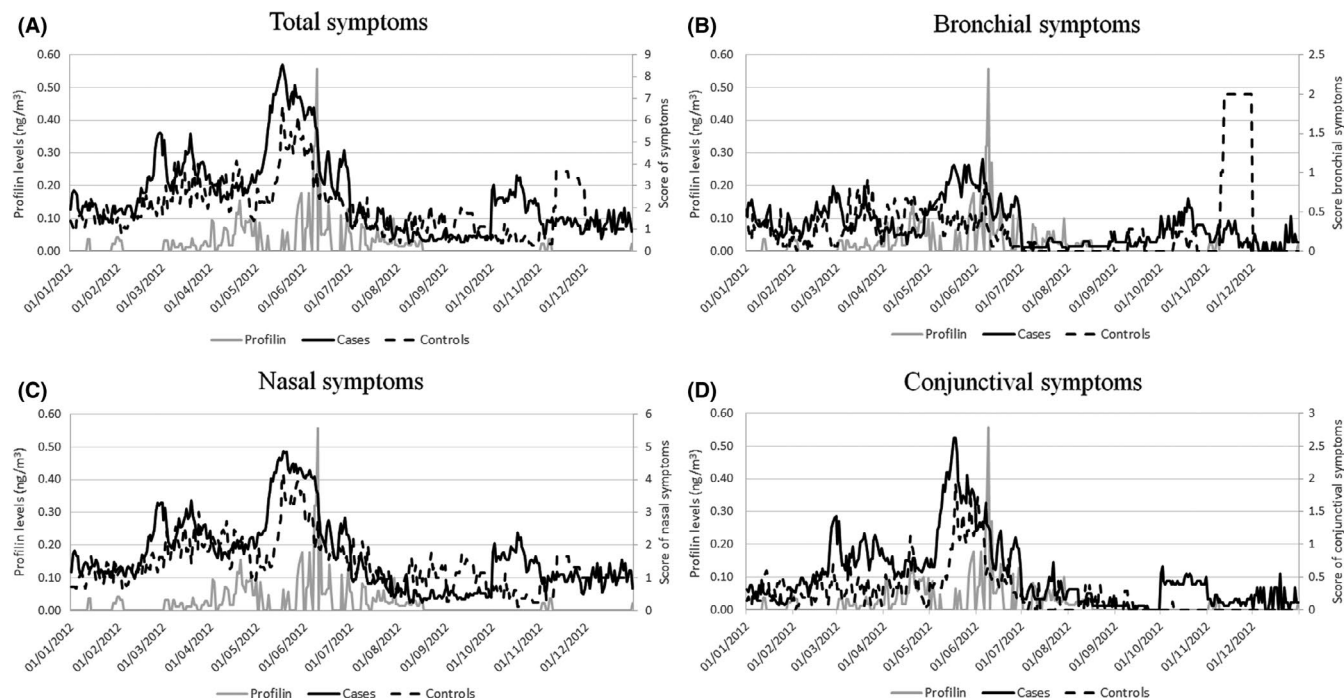


FIGURE 2 Correlation between profilin counts and clinical symptoms throughout the year. A total of 79 patients ($n = 51$ cases and $n = 28$ controls) recorded their symptoms on diary cards. (A) Total symptoms ($P < .001$); (B) Bronchial symptoms (NS along the year); significance limited to the presence of profilin in the environment ($P = .02$); (C) Nasal symptoms ($P < .001$); and (D) Conjunctival symptoms ($P < .001$)

studies,²⁸ which demonstrated this ability by positive conjunctival challenge with nPho d 2 in 65% (11/17) of patients sensitized to profilin. Ruiz-García *et al*⁸ observed that profilin was also capable of producing respiratory symptoms by bronchial challenges with nPho d 2, positive in 77% of sensitized patients. Recently published studies have also confirmed the role of profilin from *Phleum pratense* by inducing a T-cell response.²⁹

Using our technique, the highest profilin concentration was 0.56 ng/m^3 of air (10th of June), which is much smaller than the dose needed to produce symptoms ($0.3 \text{ } \mu\text{g}$ of profilin). Based on this, it is necessary to correlate the concentration of profilin in the environment with the symptoms that our patients experienced in real life, as previously described for other allergens.^{24,30} Differences in respiratory symptoms were observed between the two groups in the presence of profilin ($P = .02$), confirming the implication of profilin in the symptoms.

Regarding our patient population, the selection was performed by skin testing with purified profilin. Diagnosis was confirmed by sIgE with rBet v 2, rPhl p 12 or immunoblot in 42 patients (82.3%), although some discrepancies were observed between *in vivo* and *in vitro* diagnostics, probably explained by limitations of the *in vitro* techniques. A positive bronchial challenge was obtained in patients with negative *in vitro* diagnosis. This result suggests that skin prick test with purified allergens is a useful tool for the selection of allergic patients.

Until now, there have been only a few published cases of genuine profilin-sensitized patients.^{31,32} In our real-life study, we found that our patients sensitized to profilin had a significantly higher intensity of symptoms than those not sensitized to profilin, although both groups were polysensitized. We also observed a significantly higher risk of

developing ocular, nasal and bronchial symptoms in profilin-sensitized individuals. Both risks and intensity corroborate the idea that profilin can be a marker of severity. On the other hand, profilin may also be a marker of evolution of asthma. In our population, we observed that profilin-sensitized individuals showed twice the time of evolution of asthma symptoms (5.8 ± 4.9 vs. 2.9 ± 2.5 years) ($P < .05$). These results are in agreement with previous observations, which have suggested the importance of profilin as a marker of evolution,³³ early marker of severity¹³ or a marker of symptom severity.^{33,34} Prospective studies may clarify the role of profilin as a marker of allergic disease.

Exclusive monosensitization to profilin is not frequent. However, treatment of these patients or the majority allergic to profilins remains unsolved. Further studies will be necessary to determine whether an aetiological approach to this panallergen is possible, as has been proposed with other panallergens such as LTP.^{35,36}

In summary, our study demonstrates that environmental profilin is able to induce a specific allergic response at the respiratory level evidenced by bronchial and conjunctival challenges. In addition, patients sensitized to this panallergen showed more symptoms and were more likely to have symptoms. Therefore, sensitization to profilin can be considered a marker of severity in patients with rhinoconjunctivitis and asthma due to pollen allergy. More studies are necessary to identify the best approach for treating these patients.

CONFLICT OF INTEREST

JRH received a grant from SEAIC Foundation during the conduct of the study; MAL and JC are employees of Laboratorios LETI SLU.

AUTHOR CONTRIBUTIONS

JRH, AH, SB and MS recruited the patients and performed in vivo studies with the patients. MAL quantified the profilin and performed the in vitro assays. IM and MAL analysed data. JRH, MAL, PBJ and JC designed the study. JRH, MAL and JC wrote the manuscript.

ORCID

Jerónimo Carnés  <https://orcid.org/0000-0003-4050-2577>

REFERENCES

- Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergen. *J Exp Med* 1992;175:337-385.
- Gunning PW, Ghoshdastider U, Whitaker S, Popp D, Robinson RC. The evolution of compositionally and functionally distinct actin filaments. *J Cell Sci*. 2015;128:2009-2019.
- Wopfner N, Gruber P, Wallner M, et al. Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy*. 2008;63:872-881.
- Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? *Int Arch Allergy Immunol*. 2011;155:191-204.
- López-Torrejón G, Díaz-Perales A, Rodríguez J, et al. An experimental and modeling-based approach to locate IgE epitopes of plant profilin allergens. *J Allergy Clin Immunol*. 2007;119:1481-1488.
- Barderas R, Villalba M, Pascual CY, Batanero E, Rodríguez R. Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of *Chenopodium album* pollen: Isolation, aminoacid sequences, and immunologic properties. *J Allergy Clin Immunol*. 2004;113:1192-1198.
- Asero R, Monslave R, Barber D. Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. *Clin Exp Allergy*. 2008;38:1033-1037.
- Ruiz-García M, García del Potro M, Fernández-Nieto M, Barber D, Jimeno-Nogales L, Sastre J. Profilin, a relevant aeroallergen. *J Allergy Clin Immunol*. 2011;128:416-418.
- Asero R, Wopfner N, Gruber P, Gadermaier G, Ferreira F. Artemisia and Ambrosia hypersensitivity: co-sensitization or co-recognition? *Clin Exp Allergy*. 2006;36:658-665.
- Wensing M, Akkerdaas JH, van Leeuwen WAstrid, et al. IgE to Bet v 1, and profilin: cross-reactivity patterns and clinical relevance. *J Allergy Clin Immunol*. 2002;110:435-442.
- Barber HD. Could Profilin Be a 'Canary in a Coal Mine' of the Increasing Allergy Epidemic? *Int Arch Allergy Immunol*. 2015;168:1-2.
- Rodríguez Del Río P, Díaz-Perales A, Sánchez-García S, et al. Profilin, a change in the paradigm. *J Invest Allergol Clin Immunol*. 2018;28:1-12.
- Asero R, Tripodi S, Dondi A, et al. Prevalence and clinical relevance of IgE sensitization to profilin in childhood. A multicenter study. *Int Arch Allergy Immunol*. 2015;168:25-31.
- Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol*. 2010;6:1.
- Sastre J, Rodríguez F, Campo P, Laffond E, Marín A, Alonso MD. Adverse reactions to immunotherapy are associated with different patterns of sensitization to grass allergens. *Allergy*. 2015;70:598-600.
- Feo Brito F, Gimeno PM, Carnés J, et al. *Olea europaea* pollen counts and aeroallergen levels predict clinical symptoms in patients allergic to olive pollen. *Ann Allergy Asthma Immunol*. 2011;106:146-152.
- D'Amato G, Gentili M, Russo M, et al. Detection of *Parietaria judaica* airborne allergenic activity: comparison between immunochemical and morphological methods including clinical evaluation. *Clin Exp Allergy*. 1994;24:566-574.
- Moya R, Rubio V, Beitia JM, Carnés J, López-Matas MA. Purification and immunochemical characterization of Pla I 2, the profilin from *Plantago lanceolata*. *Mol Immunol*. 2017;83:100-106.
- Möller C, Björkstén B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. *Allergy*. 1984;39:37-41.
- Fauquert J-L, Jedrzejczak-Czechowicz M, Rondon C, et al. Conjunctival allergen provocation test: guidelines for daily practice. *Allergy*. 2017;72:43-54.
- Diamant Z, Gauvreau GM, Cockcroft DW, et al. Inhaled allergen bronchoprovocation tests. *J Allergy Clin Immunol*. 2013;132:1045-1055.
- Melillo G, Bonini S, Cocco G, et al. EAACI provocation tests with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. *Allergy*. 1997;52:1-35.
- Swanson MC, Agarwal MK, Reed CE. An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol*. 1985;76:724-729.
- Feo Brito F, Mur Gimeno P, Carnés J, et al. Grass pollen, aeroallergens, and clinical symptoms in Ciudad Real Spain. *J Invest Allergol Clin Immunol*. 2010;20:295-302.
- Feo Brito F, Alonso AM, Carnés J, et al. Correlation between Alt a 1 levels and clinical symptoms in *Alternaria alternata*-monosensitized patients. *J Invest Allergol Clin Immunol*. 2012;22:154-159.
- Wölbing F, Kunz J, Kempf WE, Grimm C, Fischer J, Biedermann T. The clinical relevance of birch pollen profilin cross-reactivity in sensitized patients. *Allergy*. 2017;72:562-569.
- Obeso D, Mera-Berriatua L, Rodríguez-Coira J, et al. Multi-omics analysis points to altered platelet functions in severe food-associated respiratory allergy. *Allergy*. 2018;73:2137-2149.
- Núñez R, Carballada F, Lombardero M, Jimeno L, Boquete M. Profilin as an aeroallergen by means of conjunctival allergen challenge with purified date palm profilin. *Int Arch Allergy Immunol*. 2012;158:115-119.
- Lund G, Brand S, Ramos T, et al. Strong and frequent T-cell responses to the minor allergen Phl p 12 in Spanish patients IgE-sensitized to Profilins. *Allergy*. 2018;73:1013-1021.
- Fernández-González D, González-Parrado Z, Vega-Maray AM, et al. *Platanus* pollen allergen, Pla a 1: quantification in the atmosphere and influence on a sensitizing population. *Clin Exp Allergy*. 2010;40:1701-1708.
- Metz Favre C, Pauli G, Castro L, Valenta R, De Blay F. Bet v 2 Responsibility in Birch Induced Symptoms. *J Allergy Ther*. 2014;5:169-170.
- Asero R, Villalta D. Profilin may be a primary airborne sensitizer: a case report. *J Invest Allergol Clin Immunol*. 2013;23:134-135.
- Barber D, de la Torre F, Feo F, et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy*. 2008;63:1550-1558.
- Alvarado MI, Jimeno L, De La Torre F, et al. Profilin as a severe food allergen in allergic patients overexposed to grass pollen. *Allergy*. 2014;69:1610-1616.
- Rodríguez MJ, Mascaraque A, Ramos-Soriano J, et al. Pru p 3-Epitope-based sublingual immunotherapy in a murine model for the treatment of peach allergy. *Mol Nutr Food Res*. 2017;61(10):1700110
- Gomez F, Bogas G, Gonzalez M, et al. The clinical and immunological effects of Pru p 3 sublingual immunotherapy on peach and peanut allergy in patients with systemic reactions. *Clin Exp Allergy* 2017;47:339-350.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ruiz-Hornillos J, López-Matas MA, Berges Jimeno P, et al. Profilin is a marker of severity in allergic respiratory diseases. *Allergy*. 2019;00:1-9. <https://doi.org/10.1111/all.14140>

Patient code

Symptom assessment criteria ¹

¹Write each day in the corresponding box 0, 1, 2, or 3, depending on the severity of each symptom:

0: no symptoms

2: moderate symptoms (annoying, but not disabling or intolerable)

1: mild symptoms (clearly present but not annoying)

3: severe symptoms (serious, disabling and / or intolerable)

MONTH (Use a sheet per month)

SYMPTOM	Day															Day															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Sneeze																															
Hydrorrhea																															
Nasal obstruction																															
Nasal itching																															
Ocular itching																															
Tearing																															
Red eyes																															
Cough																															
Chest tightness																															
Shortness of breath																															

Figure 1 - Supplementary Information: Symptom diary card.

Table 1-Supplementary Information. Additional symptoms during the conjunctival challenges.

	Result	Final concentration	Ocular pruritus	Chemosis	Episcleral redness	Tearing	Final score	Adverse effects
Case-13	Pos	3	13	3	3	3	22	S,R, PP, IOE,
Case-15	Pos	0.3	4	2	3	0	9	IOE
Case-20	Pos	0.03	2	0	2	1	5	No
Case-27	Pos	1.5	7	0	2	2	11	E
Case-31	Neg	3*	0	0	0	0	0	No
Case-33	Pos	0.3	4	2	2	0	8	IOE, E
Case-34	Pos	0.3	3	2	2	0	7	E, R
Case-38	Pos	0.3	4	0	4	0	8	No
Case-39	Pos	0.3	3	3	2	0	8	No
Case-46	Pos	1.5	8	1	3	1	13	No
Control-6	Neg	3*	2	0	0	0	2	IOE
Control-7	Neg	3*	1	1	0	0	2	No
Control-8	Neg	3*	0	0	0	0	0	No
Control-13	Neg	3*	0	0	0	0	0	N
Control-25	Neg	3*	0	0	2	0	3	No

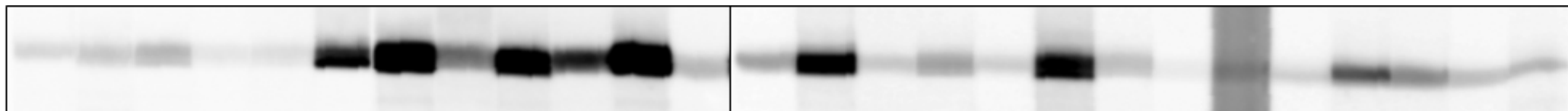
Pos: Positive result; Neg: Negative result; *: No induction of positive response; S: sneezing, R: rhinorrhea, PP palatal pruritus, ICE: itching in contralateral eye.

Table 2 – Supplementary Information. Additional symptoms during the bronchial challenges.

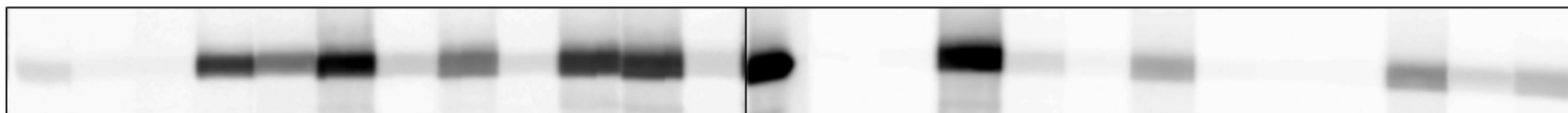
Cases	Final concentration of profilin (µg/ml)	Result	Additional symptoms
Case-2	5	Pos	NO, RN, S, D
Case -3	5	Pos	NS
Case -12	10	Pos	IT, D, C
Case -13	5	Pos	NS
Case -15	20	Neg	NS
Case -16	2.5	Pos	NO, RN, S,
Case -17	0.31	Pos	C, N
Case -21	10	Pos	NO, P
Case -22	1.21	Pos	OS, S
Case -24	20	Pos	NS
Case -26	10	Pos	IT, S, D
Case -27	20	Pos	IT
Case -31	2.5	Pos	NS
Case -32	2.5	Pos	NS
Case -33	1.25	Pos	NO, RN, S, OS
Case -34	2.5	Pos	IT
Case -35	20	Pos	S
Case -38	1.25	Pos	NS
Case -48	1.25	Pos	NO, RN, S,
Case -51	5	Pos	NS
Control-3	20	Neg	NS
Control-4	20	Neg	RN
Control-8	20	Neg	NS

Control-11	20	Neg	NS
Control-12	20	Neg	NS
Control-13	20	Neg	NS
Control-15	20	Neg	NS
Control-23	20	Neg	NS
Control-25	20	Neg	NS
Control-28	20	Neg	NS

Pos: Positive result; Neg: Negative result. NS: No Symptoms; NO: Nasal Obstruction; RN: Runny Nose; S: Sneezing; C: Cough; D: Dyspnoea; N: Nausea; P: Presyncope; OS: Ocular Symptoms; IT: Itching in the lips, mouth, and/or pharynx.



Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
rPhl p 12	0.5	1.0	0.8	N	0.6	1.8	5.5	2.7	4.6	3.7	8.9	0.6	6.6	24.0	0.5	5.1	1.7	21.7	2.0	N	4.4	N	9.2	3.1	0.5	1.9
rBet v 2	0.8	0.7	0.8	0.4	0.7	2.3	8.3	4.9	8.4	4.9	12.3	0.7	5.4	34.3	0.4	7.6	1.6	41.7	2.5	N	4.0	0.8	10.9	4.5	0.7	1.8
Wheal s.	71	25	19	27	61	17	89	48	36	48	33	30	31	57	28	64	30	139	53	19	14	42	25	28	24	55



Patient	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
rPhl p 12	0.8	N	N	7.1	2.6	9.0	1.0	4.9	N	3.8	6.6	0.7	14.5	N	N	65.3	1.3	N	1.2	N	N	N	10.6	1.3	2.5
rBet v 2	0.9	N	N	7.8	2.3	10.3	1.6	5.3	N	5.1	16.2	1.2	20.7	N	N	55.1	2.5	N	1.4	0.4	N	N	17.1	1.6	6.0
Wheal s.	14	10	41	52	49	31	41	63	52	17	24	78	28	19	7	37	39	21	41	18	19	17	73	43	26

Figure 2 - Supplementary Information: Immunoblots with the individual serum samples. Two micrograms of purified Pho d 2 were run in the solid phase. Patients sera were diluted 1:1. sIgE to rPhl p 12 and rBet v 2 are shown for each patient and expressed in (kU/L). Wheal s. (Wheal size) obtained by skin prick test with purified profilin for each patient in mm².