Prevalence and Clinical Relevance of IgE Sensitization to Profilin in Childhood: A Multicenter Study

Riccardo Asero^a Salvatore Tripodi^b Arianna Dondi^c Andrea Di Rienzo Businco^b Ifigenia Sfika^b
Annamaria Bianchi^d Paolo Candelotti^d Carlo Caffarelli^e Carlotta Povesi Dascola^e
Giampaolo Ricci^f Elisabetta Calamelli^f Nunzia Maiello^g Michele Miraglia del Giudice^g
Tullio Frediani^h Simone Frediani^h Francesco Macri^h Matteo Moretti^h Iride Dello Iaconoⁱ
Maria Francesca Patria^j Elena Varin^k Diego Peroni^l Pasquale Comberiati^l Loredana Chini^m
Viviana Moschese^m Sandra Lucarelli^h Roberto Bernardiniⁿ Giuseppe Pingitore^o
Umberto Pelosi^p Mariangela Tosca^q Anastasia Cirisano^r Diego Faggian^s Mario Plebani^s
Carmen Verga^t Paolo Maria Matricardi^u for the Italian Pediatric Allergy Network (I-PAN)

^aAllergology Service, San Carlo Clinic, Paderno Dugnano, ^bPediatric Department and Pediatric Allergology Unit, Sandro Pertini Hospital, Rome, ^cPediatric Unit, Department for Mother and Child, Ramazzini Hospital, Carpi, ^dPediatric Unit, Mazzoni Hospital, Ascoli Piceno, ^ePediatric Department, Unit of Allergy and Immunology in Evolutive Age, Department of Clinical and Experimental Medicine, University of Parma, Parma, ^fPediatric Unit, Department of Medical and Surgical Sciences, University of Bologna, Bologna, ^gPediatric Department, Second University, Naples, ^hPediatric Department, La Sapienza University, Rome, ⁱPediatric Unit, Fatebenefratelli Hospital, Benevento, ^jPediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, and ^kPediatric Clinic 2, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, ^lPediatric Section, Department of Life and Reproduction Sciences, University of Verona, Verona, ^mPediatric Department, Policlinico of Tor Vergata, Tor Vergata University, Rome, ⁿPediatric Unit, San Giuseppe Hospital, Empoli, ^oPediatric Unit, Grassi Hospital, Rome, ^pPediatric Unit, Santa Barbara Hospital, Iglesias, ^qPulmonary Disease and Allergy Unit, G. Gaslini Hospital, Genova, ^rPediatric Unit, Crotone, ^sDepartment of Laboratory Medicine, University of Padua, Padua, and ^tASL Sa1, Salerno, Italy; ^uDepartment of Pediatric Pneumology and Immunology, Charité Medical University, Berlin, Germany

Key Words

Allergic rhinitis · Children · Component-resolved diagnosis · IgE · Oral allergy syndrome · Panallergens · Pollen · Profilin

Abstract

Background: Little is known about the prevalence and clinical relevance of hypersensitivity to the plant panallergen profilin in children. **Objectives:** The present study aimed to investigate prevalence, risk factors and clinical relevance of

profilin sensitization in a large cohort of Italian children of different ages living in different geographic areas. **Methods:** Children with pollen allergy enrolled by 16 pediatric outpatient clinics sited in three main geographic areas of Italy were studied. SPT were carried out with commercial pollen extracts and a commercial purified date palm pollen profilin. IgE specific for allergenic pollen molecules, Phl p 12 (grass

R.A. and S.T. contributed equally to this study as first authors.

Abbreviations used in this paper

AR	Allergic rhinitis
ARIA	Allergic rhinitis and its impact on asthma
CI	Confidence interval
GINA	Global Initiative for Asthma
I-PAN	Italian Pediatric Allergy Network
ISAAC	International Study of Allergy and Asthma in Childhood
OAS	Oral allergy syndrome
PAN-PED	Panallergens in pediatrics
SD	Standard deviation
SIT	Specific immunotherapy
SPT	Skin prick test

profilin) and Pru p 3 (peach lipid transfer protein) were tested by ImmunoCAP FEIA. **Results:** IgE to Phl p 12 (≥ 0.35 kU/l) was observed in 296 of the 1,271 participants (23%), including 17 of the 108 (16%) preschool children. Profilin SPT was positive (≥ 3 mm) in 320/1,271 (25%) participants. The two diagnostic methods were concordant in 1,151 (91%, $p < 0.0001$) cases. Phl p 12 IgE prevalence declined from northern to southern Italy and was directly associated with IgE to Phl p 1 and/or Phl p 5 and Ole e 1. Among children with IgE to Phl p 12, OAS was provoked by kiwi, melon, watermelon, banana, apricot and cucumber. **Conclusions:** Profilin sensitization is very frequent among pollen-allergic children, occurs at a very young age and contributes to the development of childhood OAS with a typical pattern of offending foods. Pediatricians should always consider IgE sensitization to profilin while examining pollen-allergic children, even if they are at preschool age.

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Introduction

Profilin, a highly conserved 12- to 15-kDa protein present in all eukaryotic cells, is a major cause of cross-sensitization between different pollens and between pollen and plant-derived foods [1–4]. It is considered a minor pollen allergen, as it has been reported that only 10–20% of patients with pollen allergy are sensitized to this protein [2, 3, 5]. However, profilin sensitization seems to be on the rise, as shown by a more recent study that found a prevalence of 30% among an unselected population of pollen-allergic adults [6], and as suggested also by the markedly increasing prevalence of patients showing multiple pollen sensitization in in vivo or in vitro routine diagnostic tests. Profilin sensitization may ren-

der the diagnosis of seasonal airborne allergy complex (or even unfeasible) if proper in vitro diagnostic tests using recombinant allergens are unavailable and, hence, lead to errors in the prescription of allergen-specific immunotherapy [7–13]. Profilin appears to be able to act as a primary airborne sensitizer in rare cases [8], and it is a clinically relevant food allergen which is much more frequent than supposed in the past [6]. Profilin sensitization in children of different ages has been studied so far mainly with a cross-sectional approach, showing an association between age and prevalence [9, 10]. Accordingly, a longitudinal study has shown that IgE to profilin appears at a late disease stage in German children following IgE sensitization to grass-specific major allergen proteins [11]. The aim of the present study was to investigate the prevalence, risk factors and clinical relevance of profilin sensitization in a large population of children of different ages living in different geographic areas of Italy.

Patients and Methods

Study Population

The study population corresponded to that enrolled in the first Italian nationwide observational multicenter survey carried out by I-PAN to investigate the impact of sensitization to highly cross-reacting allergenic pollen molecules in childhood [12, 13]. Briefly, children were enrolled between May 2009 and June 2011 by 16 pediatric outpatient clinics in 14 Italian cities scattered throughout the country and sited in three geographic areas: northern, central, and southern Italy with its islands. Criteria for eligibility were: (a) age 4–18 years; (b) a history of pollen-induced AR and/or asthma in one of the two last pollen seasons, and (c) a positive SPT for the relevant pollen extracts. Exclusion criteria were: (1) previous specific immunotherapy for any pollen allergen and (2) any other severe chronic disease. The parents of the children recruited answered internationally validated questionnaires (ISAAC, ARIA and GINA), and a diagnosis of pollen allergy was made in the presence of: (1) nasal and/or eye symptoms for at least 3 weeks during one of the two last pollen seasons and (2) a positive SPT (wheal reaction of 3 mm or more) in accordance with clinical history and local pollen season (online suppl. fig. e1; for all online supplementary material, see www.karger.com/doi/10.1159/000441222). Further, the patients' parents (and patients themselves in the case of older children) were thoroughly interviewed about the presence of immediate itching of the oral mucosa, with or without edema of the lips or tongue, immediately following the ingestion of fresh fruits and vegetables, and the indication of trigger foods from a specific list [14]. Pollen-induced AR was classified as mild or moderate/severe as well as intermittent or persistent according to the ARIA classification. An informatics platform (AllergyCARD™; TPS Production, Rome, Italy) was used for data input. The study design and procedures were approved by the ethical committee of each participating center. Parents or tutors of all participants gave a written informed consent to clinical investigations.

Table 1. Characteristics of the study population in relation to IgE sensitization to Phl p 12

Characteristics	Without IgE to Phl p 12	With IgE to Phl p 12	p value
Males	658 (67)	208 (70)	0.368
Age, years	10.2±3.4	11.1±3.4	<0.001
Familial atopy			
Father	381 (39)	118 (40)	0.808
Mother	426 (44)	135 (46)	0.561
Geographic area			<0.001
Northern Italy	275 (28)	120 (41)	
Central Italy	464 (48)	134 (45)	
Southern Italy with its islands	236 (24)	42 (14)	
AR			0.240
Mild intermittent	247 (25)	81 (27)	
Mild persistent	105 (11)	26 (9)	
Moderate-severe intermittent	234 (24)	58 (20)	
Moderate-severe persistent	389 (40)	131 (44)	
Number of months with symptoms	4.3±1.8	4.2±1.8	0.169
Number of months in seasons with symptoms	3.9±1.4	3.9±1.4	0.888
Disease duration, years	4.95±3.2	5.77±3.5	<0.001
Asthma	358 (37)	124 (42)	0.108
OAS	203 (21)	97 (33)	<0.001

n (%) or means ± SD. The χ^2 test was used to compare frequencies and the t test to compare means.

Skin Prick Testing

Patients underwent SPT with a panel of commercial pollen extracts (ALK-Abelló, Italy) including timothy grass, olive, cypress, mugwort, pellitory, birch and hazel. Further, a commercial purified date palm pollen profilin preparation (ALK-Abelló; 50 µg/ml) was tested as well. In order to produce this diagnostic tool, natural profilin (Pho d 2) was purified from date palm extract by affinity chromatography with poly-L-proline Sepharose; purity, checked using sodium dodecyl sulfate polyacrylamide-gel electrophoresis and mass spectrometry, and amino acid analysis was found to be 99% [15, 16]. In previous studies [16, 17], one of which was carried out in northern Italy (an area where date palm is not present), this extract was at least as sensitive as Phl p 12 in identifying a large number of profilin-sensitized patients, and showed a high positive (>80%) and negative (>90%) concordance with the corresponding panallergen detected in vitro (Phl p 12). Although this extract might theoretically contain also specific palm allergens (other than profilin), to date no case of skin reactivity in the absence of polysensitization to seasonal allergens has been detected in hundreds of both children and adults tested. This extract will be referred to as palm profilin extract hereafter. Histamine (1 mg/ml) and glycerol solution were the positive and negative controls, respectively. Morrow Brown needles were used to prick the skin. Readings were taken at 15 min and wheal reactions ≥3 mm were regarded as positive.

IgE Assays

IgE for allergenic molecules was tested in the sera of patients showing a wheal reaction ≥2 mm elicited by the corresponding

allergenic source by ImmunoCAP FEIA (ThermoFisher Scientific, Sweden). The following markers of primary pollen sensitization were measured: rPhl p 1 and rPhl p 5 (grass), rOle e 1 (olive), nCup a 1 (cypress), rBet v 1 (birch), rPar j 2 (pellitory) and nArt v1 (mugwort). Further, rPhl p 12 (grass profilin), rBet v 2 (birch profilin) and rPru p 3 (peach lipid transfer protein) IgE levels were measured as well (results were expressed in kU/l). Although the current technical threshold of ImmunoCAP FEIA is 0.1 kU/l, in order to increase the specificity of in vitro tests, only levels exceeding 0.35 kU/l were considered positive.

Statistics

Data were summarized as numbers and frequencies if they were categorical and as means and standard deviations if quantitative. The χ^2 test was used to evaluate differences between the geographical areas. The χ^2 test was also applied to compare frequencies and Student's t test to compare means. Normal distribution was checked by the Shapiro-Wilk test and graphic methods. The logarithmic transformation of IgE levels was applied and geometric means presented. κ statistics were calculated to measure agreement between IgE and SPT. Multiple stepwise logistic regressions were applied to evaluate possible risk factors for sensitization to profilin in the whole population and to evaluate possible risk factors for OAS within profilin reactors. Only factors associated at univariate level ($p < 0.10$) were included in this model. Adjusted odds ratios and their 95% confidence intervals were calculated. A p value <0.05 was considered statistically significant. STATA 12.1 was used for all analyses.

Fig. 1. Prevalence of IgE seropositivity (>0.35 kU/l) to Phl p 12 in 1,271 Italian children with seasonal allergic rhinoconjunctivitis by age (a) and number of positive SPTs to unrelated pollen sources (b).

Results

Study Population and IgE Sensitization to Profilin

A total of 1,271 patients (68% males, mean age 10.4 ± 3.4) completed the present survey. IgE to Phl p 12 scored positive in 296 cases (23%). SPTs to the date palm pollen profilin were positive in 320 (25%) subjects. The two diagnostic methods gave a concordant response in 1,151/1,271 (91%, $\kappa = 0.74$, $p < 0.0001$) cases. Forty-eight patients showed IgE to Phl p 12 in the absence of skin reactivity to the date palm pollen extract, whose normally distributed level, however, was slightly lower than in their peers positive to palm SPT extract (2.16 ± 3.5 vs. 3.1 ± 3.5 kU/l, $p = 0.08$). Seventy-two patients reacted to date palm extract in the absence of detectable Phl p 12 IgE levels; in most cases (53%), these patients were characterized by borderline skin responses (wheal diameter between 3 and 3.5 mm) to the date palm pollen extract. Mean Phl p 12

Table 2. IgE to genuine pollen allergenic molecules by IgE to Phl p 12

	No IgE to Phl p 12 (n = 975)	With IgE to Phl p 12 (n = 296)	p value χ^2 test
Phl p 1 and/or Phl p 5	783 (80%)	278 (94%)	<0.001
Bet v 1	208 (21%)	92 (31%)	0.001
Ole e 1	506 (52%)	223 (75%)	<0.001
Cup a 1	306 (31%)	117 (40%)	0.01
Art v 1	73 (7%)	45 (15%)	<0.001
Par j 2	250 (26%)	55 (19%)	0.013

Frequency of IgE sensitization to major allergenic molecules of six different pollen sources among children with or without IgE to Phl p 12.

IgE levels showed a marked declining gradient from northern to southern Italy (table 1). Phl p 12 IgE levels were significantly higher among truly polysensitized subjects (defined as subjects sensitized to genuine allergens of distinct pollen sources) than among monosensitized ones (7.49 vs. 4.6 kU/l; $p = 0.01$). Only 8 patients scoring positive for Phl p 12 did not show detectable levels of IgE specific for genuine grass pollen allergens.

Characteristics and Risk Factors of IgE Sensitization to Profilin

Although profilin sensitization was detected in many preschool age children, on average patients with IgE to Phl p 12 (hereafter defined also as 'profilin reactors') were older and showed a significantly longer disease duration than patients lacking IgE to Phl p 12 (fig. 1; table 1). Age and disease duration were strongly interrelated in this population sample ($r = 0.64$; $p < 0.001$) and it was impossible to discriminate their respective role in sensitization to profilin. Profilin reactors lived more frequently in the Po valley and in central Italy, rather than on the Tyrrhenian coast, and southern Italy with its islands. Profilin reactors were also more frequently affected by OAS (table 1). Phl p 12- and Bet v 2-specific IgE levels were strongly correlated ($r = 0.94$; $p < 0.001$) and Phl p 12 IgE levels were also significantly correlated to total IgE levels ($r = 0.39$; $p < 0.001$; online suppl. fig. 2). Sensitization to profilin was significantly associated with a higher prevalence of sensitization to several genuine markers of sensitization to several pollen sources, including Phl p 1 and/or Phl p 5, Cup a 1, Art v 1 and Ole e 1; by contrast, sensitization to Par j 2 (pellitory) was inversely related with profilin sensitization (table 2; online

Table 3. Multivariate analysis of the association of Phl p 12 seropositivity with risk factors

	OR ¹	95% CI	p value
IgE reactivity to Pru p 3 (≥0.35 kU/l)	1.85	1.0–3.4	0.047
Male	0.49	0.3–0.9	0.022
Mother with OAS	4.02	1.2–13.1	0.021
Overall SPT reactivity to pollens	1.02	1.01–1.04	<0.001

¹ Adjusted for each other.

suppl. table e1). The same associations were found for SPT with the purified date palm pollen profilin (data not shown).

Food and Risk Factors Linked to OAS in Profilin Reactors

Overall, 97/296 (33%) profilin reactors reported to have experienced typical OAS (immediate oral itching with or without angioedema of the lips and/or tongue) following the ingestion of at least one plant-derived food. Another 203 children reported OAS without being sensitized to Phl p 12. Among profilin reactors, OAS was associated with older age, female gender, a mother affected by OAS and stronger atopic sensitization. In addition, profilin reactors with OAS were also more frequently affected by other allergic comorbidities (online suppl. table e2). In multivariate analysis, the strongest risk factor associated with OAS among profilin reactors was maternal OAS. Other risk factors of OAS among profilin reactors were the presence of IgE to Pru p 3, female gender and a higher intensity of atopic sensitization (table 3). Among patients with OAS, IgE reactivity to profilin was associated with OAS triggered by ingestion of kiwi, melon, watermelon, banana, apricot and cucumber, whereas the reported reactivity to the other examined food did not differ between the two groups (online suppl. table e3). This finding was confirmed analyzing the children whose profilin sensitization was not associated with sensitization to other OAS-associated molecules, including rPru p 3 and Bet v 1 (table 3).

Discussion

In a nationwide population of Italian children with seasonal AR we found a very high rate of IgE sensitization to profilin. This sensitization shows the following charac-

teristics: (1) it is quite frequent (>15%) at preschool (<6 years) age and its rate increases with age and disease duration; (2) it has a north-south gradient and is linked to multiple sensitizations to genuine pollen allergenic molecules; (3) it is associated with OAS triggered by melon, watermelon, banana, cucumber and apricot. To our knowledge, this is the first large cross-sectional epidemiological study investigating the prevalence, risk factors and clinical relevance of sensitization to the plant panallergen profilin in children.

The high prevalence of IgE sensitization to profilin found in our study group is not far from the one observed in adults [18, 19]. Moreover, IgE to profilin was also quite frequent in preschool children and tended to increase with age. This finding is in keeping with previous observations in German children [11] showing that sensitization to Phl p 12, the grass profilin, occurs only sometime after the initial grass pollen sensitization that invariably starts with the major allergen Phl p 1, followed by the other major allergen Phl p 5. The later occurrence of profilin sensitization in genetically predisposed individuals might depend on a lower concentration of this allergen in pollen or to its lower intrinsic allergenicity. Nonetheless, we also found that more than 15% of preschool age children (4–6 years) showed profilin-specific IgE, suggesting that in children showing an early onset of seasonal AR, sensitization to this panallergen may occur at a very young age. Early IgE sensitization to profilin (a relatively ‘weak’ sensitizer) [19] might thus be an early biomarker of a strong atopic predisposition and therefore imply a more severe clinical history of pollen allergy. Longitudinal cohort studies are needed to test this interesting hypothesis.

The north-south declining gradient in the prevalence of IgE sensitization to profilin can be easily explained by the similar geographical gradient in the relative importance of pollen sources, inducing IgE sensitization to this panallergen [19]. Grass, birch and ragweed pollen (all important inducers of profilin sensitization [20]) are the major pollen allergenic sources in northern Italian regions, while pellitory (a weed whose pollen contains a profilin showing little cross-reactivity with the homologous allergens present in other pollen sources) is a frequent cause of pollen allergy on the Tyrrhenian coast, in southern Italy, Sicily and Sardinia. Accordingly, the prevalence of IgE sensitization to Par j 2 (the major allergenic protein of pellitory), was negatively correlated with profilin hypersensitivity. This observation is in keeping with the previous finding that profilin reactors frequently score negative on SPT with pellitory pollen [21–23]. The

association between profilin and grass pollinosis is supported by statistics, by the existence of profilin and grass monosensitized patients and by the fact that grass pollen counts across Italy parallel the prevalence of profilin sensitization. Although we also found an association between profilin and Ole e 1 sensitization, patients monosensitized to olive and profilin have not been reported so far, and there is published evidence that profilin sensitization is not linked to olive [24].

A clinically relevant observation of our study is that profilin sensitivity is associated with melon-, watermelon- and banana-induced OAS, which largely confirms the findings of previous studies carried out in adults [6, 25]. Of relevance, this finding was confirmed in children whose profilin sensitization was 'isolated', i.e. not associated with IgE to other OAS-inducing molecules (e.g. LTPs or PR.10). This outcome clearly demonstrates that profilin is a clinically relevant plant food allergen in childhood even at preschool age. Overall, this finding highlights the importance of testing for profilin in children showing multiple pollen sensitization, and perhaps the most important clinical implication of our study is that oral symptoms possibly suggestive of OAS have to be sought even in very young pollen-allergic children. A more comprehensive analysis of OAS, including children not sensitized to profilin, was out of the scope of this paper and will appear in a specifically designed paper that is currently in preparation.

We have to admit that our study has some limitations. First, we defined profilin sensitization based on the presence of IgE to Phl p 12 (≥ 0.35 kU/l) though Phl p 12 might not be universally representative of all profilins [26]. However, variability among profilins is very low when compared to other highly cross-reactive panallergens such as PR.10 and LTPs. Accordingly, our definition was highly concordant with the results of SPT with the date palm pollen extract highly enriched in native Pho d 2. Moreover, an excellent relationship was observed be-

tween serum levels of IgE to Phl p 12 and Bet v 2 (birch profilin). Second, in our study, the diagnosis of OAS and the information on its triggers were based on a questionnaire, but it was not confirmed by oral challenge tests with fresh foods and vegetables. Exact symptom reporting by parents may have been difficult especially in the case of preschool children. Nevertheless, the profiles of foods associated with OAS were so typical of profilin sensitization [25, 27] to conclude that patients' answers were reliable enough. Third, the examined population sample, although large enough, is not representative of the Italian population as a whole. Hence, generalizability of our results could be limited and studies in other settings and different populations will of course reflect local climatic and aerobiological conditions.

In conclusion, this study confirms the high clinical relevance of studying large cohorts of patients by recombinant allergens [17, 28], and shows that profilin sensitizes even preschool children [13, 27] and can contribute significantly to the development of food allergies due to its ubiquitous distribution in the vegetable kingdom. Given the epidemiological relevance of sensitization to profilin in childhood, the hypothesis that an early administration of pollen-specific immunotherapy may prevent sensitization to this and other annoying panallergens deserves to be investigated.

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Disclosure Statement

There are no conflicts of interest to declare.

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