

Detection of Phl p 1, Phl p 5, Phl p 7 and Phl p 12 Specific IgE Antibodies in the Sera of Children and Adult Patients Allergic to Phleum Pollen

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ABSTRACT

Background: Grasses belong to major sources of inhaled allergens. The knowledge of particular molecules responsible for hypersensitivity is of crucial importance for better understanding of individual differences among single allergic subjects and allergic populations living in various world-areas.

Methods: Specific-IgE-antibodies against Phl p 1, Phl p 5, Phl p 7, Phl p 12 were detected in a group of 130 Phleum-allergic-subjects (82 children, 48 adults).

Results: Phl p 1 antibodies were detected in most pediatric and adult patients, however, the children were associated with higher RAST classes more often. Anti-Phl p 5-antibodies were found more frequently in adults. An increase was observed in the number of pediatric patients reacting to Phl p 7 and Phl p 12. There were no differences in concentrations of specific-IgE against Phl p 5, Phl p 7 and Phl p 12 depending on age. Almost 10% of allergic children produced antibodies directed exclusively against minor allergens or did not produce specific-IgE-antibodies against tested molecules. Part of the patients reacted to profilin and calcium-binding protein originating from only one source (Phl p 12/Bet v 2 and Phl p 7/Bet v 4).

Conclusions: Antibodies against Phl p 1 and Phl p 5 can be used as a marker of allergy to grasses in adult patients. Children reacted exclusively to minor allergens more frequently than adults. Prolonged allergen exposure is evidently necessary to induce sensitization to Phl p 5. A high level of homology between profilins and calcium-binding proteins enables only one allergen to be used for diagnostic purposes but a possibility of a reaction to species-bound epitopes should be taken into account.

KEY WORDS

Phleum pollen, Phl p 1, Phl p 12, Phl p 5, Phl p 7, recombinant allergens, specific IgE

INTRODUCTION

Grasses are an important source of inhaled allergens.^{1,2} One of the reasons is a great amount of pollen produced by their species together with their wide distribution in climatic zones, which are densely populated. One of the significant grass allergens is the Phleum pollen (*Phleum pratense*). Up to 20% of allergic individuals worldwide are hypersensitive just to the Phleum and related species of grasses of which numerous cross-reactivities have been reported. Pol-

len allergens from various grass species are classified into groups on the basis of their cross-reactivities.

Group I comprises allergens represented by the major allergen of the Phleum, Phl p 1. Two isoforms of a molecular weight of 35 and 37 kDa are designated as Phl p1.^{3,4} For example, pollens of wild grasses and corn (rye grass, cocksfoot, fescue grass, sweet vernal-grass and other grasses) are cross-reactive allergens.⁵ The number of Phleum pollinosis hypersensitive to group I varies between 70 to 100% in various countries in Europe.⁶ Because of the

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wide representation of group I allergens in grass pollens and a high cross-reactivity of IgE antibodies among group I allergens, the antibodies against Phl p 1 can be used as a diagnostic marker of the grass allergy.

Another major group of grass allergens is group V with allergens to which 60 to 93% of patients with pollinosis do react. Group V is represented by a Phl p 5 molecule. Two isoforms with a molecular weight of 38 kDa (Phl p 5a), 29 kDa and/or 32 kDa (Phl p 5b) are designated as Phl p 5. Each isoforms can be split at least into 4 isoallergens. An area of a magnitude of 11.2 kDa at the N-terminal fragment was found as a binding site for IgE in allergenic molecule Phl p 5a. This area that contains more IgE-binding epitopes exhibits a strong reactivity in up to 76% of Phleum allergic individuals. Another IgE-binding area was found at the C terminal-site of the protein.⁷⁻¹⁰ Phl p 5b induces allergic and asthmatic manifestations in 90% of grass pollinotics.

In addition to major allergens, specific IgE antibodies are directed also against the minor allergens Phl p 7 and Phl p 12. Phl p 7 is an intracellular pollen protein with a molecular weight of 8.6 kDa.¹¹ Antibodies against the calcium-binding protein family were detected in 5 to 10% of pollen allergic individuals. The calcium-binding protein is also responsible for cross-reactivities between pollens of grasses, trees and weeds.^{12,13}

Another group of allergenic molecules responsible for cross-reactions between various species of plants includes plant profilins designated as Phl p 12 in the Phleum pollen. Profilins are present in all eukaryotic cells of plant and animal origin. The molecular weight of plant profilins is reported to be between 12 to 15 kDa. They are highly conserved molecules with numerous homologies. The number of patients producing IgE antibodies against profilin were detected variably between 8 to 15% in Europe.⁶ Phl p 12 specific IgE antibodies account for cross-reactions with homologous profilins in many other allergens in both pollens and foods.¹⁴

The purpose of this study was to characterize the profile of specific IgE antibodies against the major (Phl p 1 and Phl p 5) and the minor (Phl p 7 and Phl p 12) Phleum allergens in the Czech Republic. Particular attention was paid to differences between the pediatric and adult populations of the Phleum pollinotics. Moreover, we focused on the reactivity of pollen allergic individuals against recombinant profilins and calcium-binding proteins from various plant species (Bet v 2 × Phl p 12 and Bet v 4 × Phl p 7).

METHODS

STUDY SUBJECTS

During the years 2004-2009, the sera of 130 patients with a case history of seasonal pollen allergy including symptoms of allergic asthma or rhinoconjunctivi-

tis were collected. The tested group comprised 67 males and 63 females of whom 82 were children aged 1-18 years, (53 boys, 29 girls) and 48 adults aged 19-70 years (14 males and 34 females). Criteria for the final selection to the present study were patient history (seasonal allergic rhinitis, rhinoconjunctivitis, sneezing), positive skin prick test to Phleum pollen, laboratory parameters (increased total IgE, positive IgE reactivity to Phleum pollen extract (g6) established by FEIA as measured by Phadia CAPsystem (UniCap250 Firm Phadia, Uppsala, Sweden). Patients exhibiting a concentration of specific IgE antibodies against Phleum pollen higher than 0.35 kU/l were evaluated as positive and included in the study. IgE antibodies against the major and minor Phleum pollen allergens were tested before the vaccination therapy.

METHODS

Serum levels of allergen-specific IgE against recombinant allergens Phl p 1, Phl p 5, Phl p 7, Phl p 12 were measured by the FEIA method (UniCap250 Firm Phadia) according to the instructions of the manufacturer. Diagnostics with birch pollen extract, recombinant, Phl p 1, Phl p 5, Phl p 7, Phl p 12 are commercially available. The evaluation was performed quantitatively in kU/l or by RAST scoring system.

Specific IgE antibodies against recombinant Bet v 2/Phl p 12 respectively Bet v 4/Phl p 7 were detected in the sera of 30 patients (16 males and 14 females) sensitized to profilin respectively 22 patients (12 males and 10 females) sensitized to calcium-binding protein. Tests were performed using FEIA (Phadia UniCap250 Firm).

All patients sera were analyzed in the laboratory of the Faculty Hospital Bulovka, Prague. All tested parameters were requested by physicians as a part of laboratory screening. The protocol was approved by the Ethical Committee of the Faculty Hospital Bulovka and included written informed consent of study subjects and data management with patient anonymity.

STATISTICS

Statistical analysis was performed using chi-square test.

RESULTS

In sera of 130 patients exhibiting IgE antibodies against the Phleum pollen (g6), IgE antibodies against the major and minor Phleum allergens Phl p 1, Phl p 5, Phl p 7 and Phl p 12 were tested.

1/SPECIFIC IgE ANTIBODIES AGAINST MAJOR AND MINOR PHLEUM ALLERGENS IN PEDIATRIC AND ADULT POPULATIONS

In the group of patients tested, the rate of occurrence of specific IgE antibodies against the major and minor allergens of the Phleum was measured. Antibod-

IgE Phleum Reactivity Related to Age

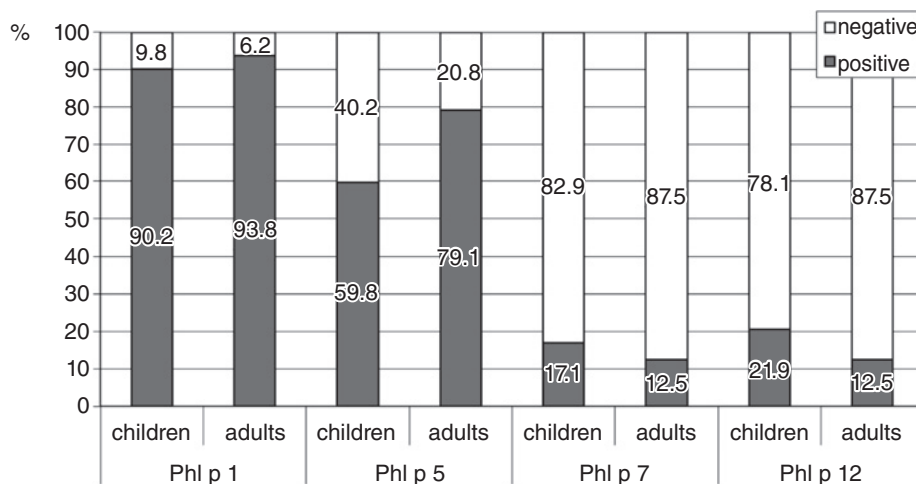


Fig. 1 Comparison of occurrence of specific IgE against major and minor Phleum pollen allergens in the pediatric and adult populations.

ies against Phl p 1 were found in the sera of 92% of the patients, 69.5% of allergic individuals reacted against the second major allergen Phl p 5, 14.8% of the patients reacted against the minor allergens Phl p 7 (calcium-binding protein), and 16.6% against Phl p 12 (profilin). Attention was focused to the differences in the rate of occurrence of specific IgE antibodies in pediatric and adult populations. Tests were performed in 82 children aged 0 to 18 and 48 adults over 18 years of age. No significant differences were found between the children and adults as for the number of patients with pollinosis reacting against Phl p 1 (90.2% respectively 93.8%) ($p = 0.714$). However, when comparing patients with the presence of IgE antibodies against Phl p 5, we found a 20% higher incidence in the group of adult patients ($p = 0.023$) (59.8% of the children, 79.1% of the adults). Antibodies against Phl p 7 were detected in 17.1% of the children and 12.5% of the adults ($p = 0.486$). Similarly, 21.9% of the children and 12.5% of the adults reacted against Phl p 12 ($p = 0.180$). The results are summarized in Figure 1.

Concentrations of specific IgE antibodies against major and minor Phleum allergens in pediatric and adult populations are summarized in Table 1. The results were evaluated by RAST classes. In children, the antibodies against Phl p 1 were evaluated by the highest RAST classes (4-6) in 39.1%, whereas in adult patients, only 25.1% of the sera tested reached these values. On the other hand, no differences were found in concentrations of specific IgE antibodies against Phl p 5 in adult pollinotics (27.1%) as compared to the tested children (23.2%). No differences between pediatric and adult pollinotics were found in concentrations of specific IgE against minor Phleum allergens.

2/MONOSENSITIZATION AND/OR POLYSENSITIZATION TO Phl p 1, Phl p 5, Phl p 7, Phl p 12 IN PEDIATRIC AND ADULT POPULATIONS

In the tested group, a spectrum of specific IgE antibodies against particular Phleum allergens was detected. 71.4% of allergic individuals reacted exclusively against major allergens. Antibodies against minor allergens were detected in 24.4% of the tested sera in addition to antibodies against major allergens.

The last group was composed of patients (8.5%) who did not exhibit detectable IgE antibodies against major allergens while having antibodies against minor allergens (3.6%), or they did not have antibodies against any of the tested molecules (4.9%), the positivity of IgE against the Phleum pollen being confirmed. This group consisted only from allergic children.

In the spectrum of specific IgE, differences were found between the group of the children and that of the adults. The results are summarized in Table 2. 65.9% of the children and 76.9% of the adults reacted exclusively against major allergens. In addition to antibodies against major allergens, antibodies against minor allergens were confirmed in 25.6% of the children and in 23.1% of the adults.

Occurrence of a high number of patients who in addition to antibodies against major allergens also have antibodies against minor allergens or do not have antibodies against major allergens at all should be taken into consideration when choosing a hyposensitization treatment. Especially in the pediatric population, the reactivity to minor allergens is relative high (34.1% of pediatric pollinotics).

3/SPECIFIC IgE ANTIBODIES AGAINST PROFILIN (Bet v 2 AND Phl p 12) AND CALCIUM-BINDING PROTEIN (Bet v 4 AND Phl p 7) FROM THE BIRCH AND PHEUM POLLEN

A high homology has been demonstrated between

Table 1 Percentage distribution of Phl p1, Phl p5, Phl p7 and Phl p12 specific-IgE (in RAST classes) in the sera of pediatric (82) and adult (48) Phleum pollen allergic subjects

RAST classes	0	1	2	3	4	5	6	4-6
Phl p 1	9.8	3.7	19.5	28	19.5	9.8	9.8	39.1
Phl p 5	40.2	6.0	9.8	20.8	11.0	7.3	4.9	23.2
Phl p 7	82.9	3.7	6.1	1.2	2.4	0	3.7	6.1
Phl p 12	78	6.1	9.8	2.4	3.7	0	0	3.7
adults								
RAST classes	0	1	2	3	4	5	6	4-6
Phl p 1	6.3	4.2	29.2	35.3	14.6	6.3	4.2	25.1
Phl p 5	20.8	6.3	20.8	25	12.5	10.4	4.2	27.1
Phl p 7	87.4	2.1	6.3	2.1	2.1	0	0	2.1
Phl p 12	87.4	6.3	0	6.3	0	0	0	0

specific IgE IU/l	RAST classes
<0.35	0
0.35-0.7	1
0.7-3.5	2
3.5-1.75	3
17.5-50	4
50-100	5
>100	6

Table 2 Monosensitization or/and polysensitization in the population of pediatric and adult Phleum pollen allergic individuals
Spectrum of Tested Allergens

children	%	adults	%
Phl p 1+ Phl p 5- Phl p 7- Phl p 12-	24.4	Phl p 1+ Phl p 5- Phl p 7- Phl p 12-	20.7
Phl p 1+ Phl p 5+ Phl p 7- Phl p 12 -	41.5	Phl p 1+ Phl p 5+ Phl p 7- Phl p 12 -	56.2
Phl p 1+ Phl p 5+ Phl p 7+ Phl p 12 -	3.7	Phl p 1+ Phl p 5+ Phl p 7+ Phl p 12 -	6.3
Phl p 1+ Phl p 5+ Phl p 7+ Phl p 12+	8.5	Phl p 1+ Phl p 5+ Phl p 7+ Phl p 12+	6.3
Phl p 1+ Phl p 5+ Phl p 7- Phl p 12 +	4.9	Phl p 1+ Phl p 5+ Phl p 7- Phl p 12 +	4.2
Phl p 1+ Phl p 5- Phl p 7- Phl p 12 +	4.9	Phl p 1+ Phl p 5- Phl p 7- Phl p 12 +	0
Phl p 1+ Phl p 5- Phl p 7+ Phl p 12+	2.4	Phl p 1+ Phl p 5- Phl p 7+ Phl p 12+	0
Phl p 1- Phl p 5+ Phl p 7- Phl p 12+	0	Phl p 1- Phl p 5+ Phl p 7- Phl p 12+	2.1
Phl p 1- Phl p 5+ Phl p 7- Phl p 12 -	1.2	Phl p 1- Phl p 5+ Phl p 7- Phl p 12 -	4.2
Phl p 1- Phl p 5- Phl p 7- Phl p 12+	1.2	Phl p 1- Phl p 5- Phl p 7- Phl p 12+	0
Phl p 1- Phl p 5- Phl p 7+ Phl p 12-	2.4	Phl p 1- Phl p 5- Phl p 7+ Phl p 12-	0
Phl p 1- Phl p 5- Phl p 7- Phl p 12-	4.9	Phl p 1- Phl p 5- Phl p 7- Phl p 12-	0

profilins and calcium-binding proteins from various species of plants because these are conserved proteins with significant biological functions. We therefore focused attention on detecting the reactivity to recombinant Bet v 2 and Bet v 4 molecules in patients carrying detectable IgE antibodies against Phl p 7 and Phl p 12. The results show that 83.3% of the patients have detectable antibodies against the profilin of both the Phleum and the birch (Bet v 2/Phl p 12). A number of patients (16.7%) synthesizes specific IgE

against profilin from one source only. Antibodies against the Bet v 4/Phl p 7 calcium-binding protein were found in the sera of 78.6% of the patients. There were 21.4% of the patients reacting exclusively against one protein. The results are summarized in Figure 2.

DISCUSSION

This study presents summarized results concerning the rate of occurrence of specific IgE antibodies

IgE Phleum Reactivity Related to Age

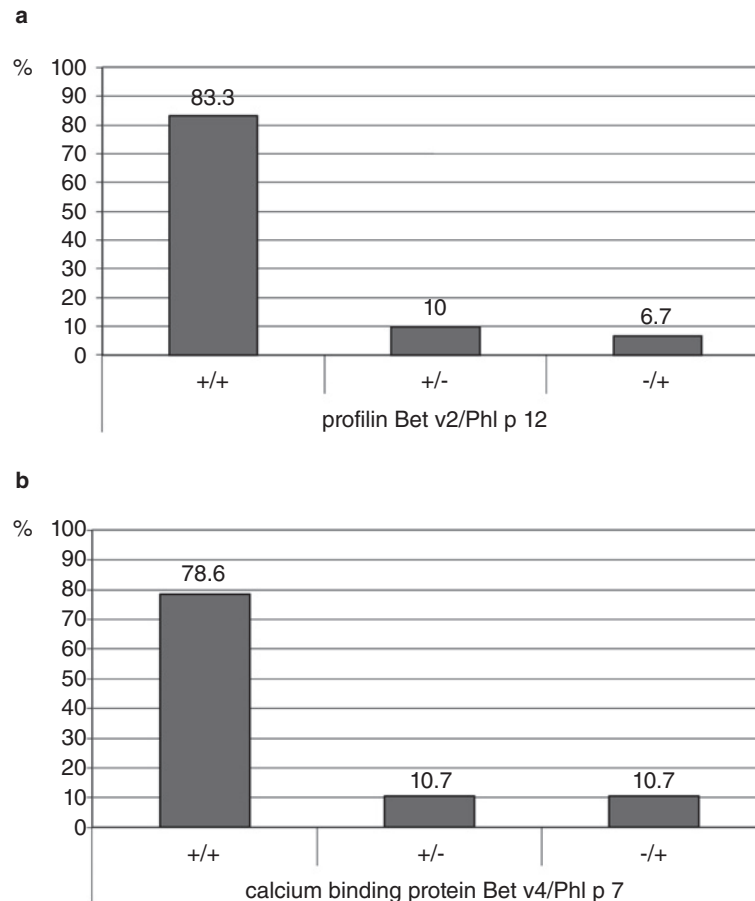


Fig. 2 Cross-reaction specific IgE among profilin and calcium-binding protein from different sources.

against major (Phl p 1, Phl p 5) and minor Phleum allergens (Phl p 7 and Phl p 12) and their concentrations in a group of Phleum pollen allergic individuals living in the Czech Republic. Although population differences in the reactivity of allergen specific IgE antibodies in dependence on various areas of Europe and the world are well documented, yet very little is known about changes of the allergenic profile of the population living in one area depending on age.^{6,15-17} Attention has therefore been focused on the differences between pediatric and adult patients regarding their capacity to pathologically react to major and minor Phleum pollen allergens.

In most patients, antibodies against Phl p 1 corresponded to the rate of occurrence of these antibodies in Phleum pollen pollinotics in Europe.⁶ When comparing pediatric and adult pollinotics we have not found a difference in the rate of occurrence of antibodies. Children, however, have been associated with higher RAST classes more frequently than adult patients. Higher concentrations of specific IgE antibodies against Phl p 1 in children correspond with results obtained by testing pediatric and adult birch pollen allergic individuals against Bet v 1, Bet v 2 and Bet v

4.¹⁸

On the basis of a comparison of pediatric and adult Phleum pollen allergic individuals we have found an increase in the number of Phl p 12 positive findings in children even when this increase was not so pronounced as in testing the Bet v 2 specific IgE antibodies in birch pollen pollinotics. It is possible that children react preferentially to profilin epitopes which are species-specific and we have therefore found certain differences in the number of allergic children reacting against Bet v 2 and Phl p 12. The results obtained by the detection of Phl p 7 specific IgE antibodies similarly show a tendency toward an increase of positive findings in pediatric age which we have already observed when evaluating Bet v 4 antibodies.¹⁸

Surprisingly, a rapidly growing number of children reacted with minor allergens responsible for cross-reactions with pollens of unrelated species of plants and plant-derived food. The reason of this higher reactivity in children is unknown. The question is whether this tendency relates to a change in the life style, particularly to an insufficient stimulation by previously common allergens, e.g., of infectious origin and to an increasing exposition of the children organ-

ism to chemical substances from environmental surroundings and foods. Furthermore, the expansion of ornamental plants and trees in densely populated areas which increased the allergenic burden of child's organism, may be responsible for growing number of polyreactive allergies. Climate changes and environmental industrialization may be another reason of pathological reaction in children. It is, however, possible to state that there is an unambiguously increasing trend to pathologically react to non-pathogenic antigens whether of an exogenic or endogenic origin (higher incidence of allergic and autoimmune disease).¹⁹⁻²⁴

It is obvious that the suitability of classical hyposensibilization therapy of these patients by vaccines standardized by the content of major allergens might be limited. Simultaneously in the patients, where vaccination does not provide a protective long-term effect, insufficient concentration of allergenic active molecules in the applied extract might be a cause.

Allergen extracts currently used in diagnostics and the subsequent hyposensibilization are obtained from natural sources. Their standardization is still problematic. Only in 1999, WHO issued a position paper concerning the standardization of allergens. A final goal is the use of extracts containing defined amounts of major allergens. Most allergen extracts are thus standardized only in the biological activity and a concentration of a major allergen, not minor allergens, however (NIBSC Biological Reference Materials 1999).

The gradually expanding knowledge of molecular and biochemical properties of allergen molecules makes it possible to prepare these proteins synthetically to obtain highly purified and precisely characterized extracts of recombinant allergens which allow "an allergenic profile" to be determined for each patient. The precise knowledge of the patient's "allergenic profile" helps to choose a better targeted immunotherapy. The selection of an appropriate allergen extract containing precisely defined amount of an individual active molecule and/or molecules might lead to optimization of immunotherapy and to reduction of adverse side effects of possible newly formed specific IgE antibodies.²⁵ The relatively high number of children with minor allergen antibodies, strengthen us in the view that allergenic profile examination should precede initiation of vaccination therapy and should become one of the criteria for the decision to initiate allergen immunotherapy. It is also the question of whether standardized vaccines for minor allergens would not be appropriate to produce.

Phl p 5 was the only recombinant allergen tested in which we have found higher sensibilization in the adult population. Likewise, we have demonstrated that adult pollinotics were evaluated by higher RAST classes more frequently than children. With respect

to the fact that in the neighbouring countries (Austria, Germany) Phl p 5 is detected in up to 90% of the patients tested, a longer-lasting allergen exposure is undoubtedly necessary for the development of sensibilization. It is possible that articles addressing the issue of allergies to Phleum involved preferentially adults since no data were shown regarding the average age of patients.⁶ Considering that when testing specific antibodies against major and minor Phleum and birch allergens we have always found the same or a significantly higher reactivity of allergic children this phenomenon should be studied further and the occurrence of Phl p 5 IgE antibodies in individual age categories should be investigated in more detail. One of the possible explanations of why a prolonged exposure is necessary before sensibilization was elicited could be a different mode of binding and internalization of Bet v 1 and Phl p 1 by mucosal Langerhans cells in contrast to Phl p 5. Bet v 1 and Phl p 1 are likely absorbed by micropinocytosis, without detectable receptor binding.²⁶ Phl p 5 is likely to bind to a receptor (e.g., FcεpsilonRI) required for its internalization. In this case, the presence of allergen-specific IgE antibodies bound to Fcεpsilon at the cell surface would be necessary for the internalization and presentation of the allergen by immunocompetent cells. There is a speculation as to whether this is a general phenomenon or whether receptor binding occurs only in internalization by Langerhans cells.²⁷ There is a question of whether various allergens are absorbed and consequently presented by immunocompetent cells by quite a different mode. This could affect not only the development of allergy but also the success of sublingual immunotherapy.

It is known that plant profilins are, due to their biological function, very conserved molecules exhibiting a high homology. Nevertheless, it has been described that up to 60% of specific IgE react with profilin epitopes which are species-specific.²⁸ A high degree of homology between particular molecules of profilins makes it possible to use one profilin for diagnostics, however, it is necessary to take into account the possibility that specific IgE can be bound to species-specific epitopes. IgE antibodies binding to species-specific epitopes of profilin are likely to be less responsible for cross-reactions with food allergens.

Calcium-binding proteins (Phl p 7 and Bet v 4), which also exhibit high homology, are strongly cross-reactive. Patients with IgE directed against epitopes of the calcium-binding protein are a group of allergic subjects exhibiting a polysensitivity against various unrelated species of plants (trees, grasses, weeds). On the other hand, significant structural differences have been identified.¹³ It can be assumed that even calcium-binding proteins contain conserved epitopes which are widely spread in many species and are responsible for the cross-reactivity of specific IgE anti-

bodies and epitopes with species-bound areas. Almost a quarter of the tested allergic subjects had specific IgE antibodies exclusively directed against one of them.

In conclusion, it can be stated that IgE antibodies against Phl p 1 and Phl p 5 might be used as a reliable marker of allergy to grasses in adult patients in particular. One part of pediatric patients reacts only to minor allergens and their future profile of allergen-specific antibodies is unclear. Whether the reactivity against minor allergens will be persist should be evaluated after several years. There is also a possibility of upregulation of specific IgE antibodies. Furthermore, some of these allergens are responsible for cross-reactivity between pollens and plant food, therefore we consider these findings as quite important with respect to an increase of food allergies in children worldwide.²⁹⁻³¹

The high degree of homology between profilins and calcium-binding proteins allows only one allergen to be used for the diagnostic purposes, however, the possibility of an allergic reactivity to species-bound epitopes needs to be taken into account with respect to a design of future vaccines.

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