

Profilin cross-reactive panallergen causes latex sensitization in the pediatric population allergic to pollen

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ABSTRACT

Background: Component-resolved diagnostics (CRD) has been demonstrated to be an excellent new tool for improving the current diagnosis of allergies, and it allows differentiation between polysensitization and cross-reactivity.

Objective: To demonstrate the role of cross-reactive pollen allergens in pediatric patients living in areas with large amounts of airborne grass pollen grains who are sensitive to grass pollen and latex.

Methods: Serum samples were obtained from 106 children between 3 and 14 years of age diagnosed with allergies to pollen based on clinical history, skin prick tests, and specific immunoglobulin E (IgE). None of them had allergy symptoms to latex or fruits. From these 106 children, 56 patients revealed positive results to *Phleum*-specific major allergens but not to cross-reactive allergens. The other 50 patients who showed positive specific IgE to *Phleum*-specific major allergens and to cross-reactive pollen allergens also showed positive results to latex allergens. CRD was carried out by specific IgE quantification using a fluoro-enzyme immunoassay (ImmunoCAP System).

Results: Results demonstrated a positive significant relationship between the specific IgE to Hev b 8 and Phl p 12 and also between the specific IgE to Hev b 8 and latex extract in the group of patients sensitized to species-specific and cross-reactive *Phleum* allergens. Positive significant relationships were also found between profilin and avocado or peach sensitizations. No other latex allergens gave positive results.

Conclusion: The apparent sensitization to latex in pediatric patients allergic to grass pollen is caused by the cross-reactive profilin panallergen; however, it appears not to be clinically relevant.

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Introduction

Sensitized individuals may develop allergic symptoms when exposed to any product containing latex. Cremer and colleagues¹ reported more than 10 years ago that the prevalence of latex allergies in children increased not only in risk groups but also in atopic children or healthy subjects. More recently, Arminda et al² reported that the prevalence of latex allergies in children is not currently increasing because powder-free and latex-poor gloves have been introduced. In these groups, data about sensitization prevalence are scarce and involve different population samples and different allergy tests. This makes comparison across different studies difficult.

The term *component-resolved diagnosis* (CRD) has been coined to designate diagnostic tests based on pure allergen molecules that are either produced by recombinant expression of allergen-encod-

ing complementary DNAs or by purification from natural allergen sources.³ This method has been demonstrated to be an excellent new concept for improving the current laboratory diagnosis of allergic diseases. The current availability of individualized allergens makes it possible to study the role of each molecule in diagnosis and prognosis and helps to solve the controversy about "polysensitization and cross-reactivity."⁴ Thus, CRD could be the appropriate tool for measuring the real sensitization prevalence of latex allergies through their specific allergens.

Recently, Quercia and colleagues⁵ and Ebo and colleagues⁶ demonstrated that CRD is a reliable tool for diagnosing latex allergies, discriminating between a genuine allergy and sensitization, and demonstrated that latex profilin is clinically irrelevant in a group of adult patients sensitized to latex.

These authors have demonstrated that panels including Hev b 5, Hev b 6, and Hev b 1 generally allow identification of most latex-allergic patients. Moreover, using a broader repertoire of components might also aid in evaluating cross-reactivity and identifying patients at risk for a latex–fruit syndrome. They could demonstrate

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that apparently irrelevant serum immunoglobulin E (sIgE) antibodies against latex generally result from sensitization for grass pollen profilin or cross-reactive carbohydrate determinant (CCD), and that recognition of these components can improve the diagnosis of latex allergy.

With this in mind, and taking into account that previously we could demonstrate that 46% of the pediatric population living in areas with a high pressure of airborne grass pollen grains⁷ and sensitized to grass pollen showed specific IgE to cross-reactive allergens from *Phleum pratense* Phl p7 (pollen panallergen: calcium-binding protein) or Phl p12 (plant allergen: profilin),⁴ the objective of this study was to demonstrate the role of a cross-reactive pollen profilin panallergen in pediatric patients sensitized to latex.

Methods

Study population

Serum samples for molecular diagnosis were obtained from 106 children between 3 and 14 years of age diagnosed with allergic rhinitis or asthma/rhinitis caused by pollen on the basis of the clinical history and skin prick tests. All patients referred respiratory (rhinitis or asthma/rhinitis) during the grass pollen season, and no children referred previous or current latex or food allergy symptoms. These patients visited the outpatient clinics of the Service of Pediatrics of "Complejo Hospitalario de León" (León, Spain) and were referred for extensive workup studies. A panel of allergens for prick test, including mites (*Dermatophagoides pteronyssinus* and *D. farinae*), pollens (grasses, *Artemisia vulgaris*, *Ambrosia elatior*, *Sal-sola kali*, *Plantago lanceolata*, *Olea europaea*, *Cupressus sempervirens*, and *Platanus acerifolia*), molds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium chrysogenum*), epithelia (dog and cat), latex, milk fractions, and egg whites and yolk (Laboratorios LETI S.L., Barcelona, Spain) was used. A standard prick test procedure was performed according to Jiménez and colleagues,⁸ with wheal diameters 3 mm or larger considered positive results. All patients were sensitized to pollen grasses and had seasonal symptoms shared with grass pollination. Fifty-six percent of the patients also had positive skin prick tests to pollen species other than grasses.

The study was performed in compliance with the Declaration of Helsinki (1964), and oral informed consent was obtained from the tutors of the patients. The research was approved by the Ethic Committee of the Complejo Asistencial de Leon (Leon, Spain).

Specific IgE quantification

Specific IgEs against pollen allergens from different species were determined according to requests issued by the pediatric consultations. Specific IgEs against recombinant individualized grass allergens based on the results of specific IgE testing against the allergenic species obtained from each of the issued requests were also quantified. Specific IgE assays were carried out using Fluoro-Enzyme Immunoassay (ImmunoCAP 250 Specific IgE, Phadia AB, Uppsala, Sweden). Allergen extracts from latex and different fruits (pineapple, apple, peach, kiwi, avocado, banana, and chestnut) and recombinant allergens of *Phleum pratense* (rPhl p 1: expansin, rPhl p 5: ribonuclease, rPhl p 7: timothy polcalcin and rPhl p 12: timothy profilin), latex (rHev b 1: latex elongation factor, rHev b 5, rHev b 6: hevein-like protein, rHev b 8: latex profilin and rHev b 11: class I chitinase), and cross-reactive carbohydrate cross-reactive determinants (Ana c 2: bromelain and Ana c 3 MUXF3) were used. Allergens from pollens other than grass pollen that were present in the environment where the patients lived (*Plantago lanceolata*, *Olea europaea*, *Chenopodium album*, *Quercus alba*, *Cupressus sempervirens*, *Ambrosia elatior*, and *Artemisia vulgaris*) were also tested. Results of 0.35 kU/L or higher were considered positive.

Statistical analysis

The statistical analysis was done using GraphPad Prism Version 4.03 software, (GraphPad Software Inc., La Jolla, California).

Comparisons between the specific IgE values to latex profilin and timothy grass profiling between latex extract and Hev b 8 or between Hev b 8 and each of the fruit extracts studied were analyzed using linear regression (r_1 and p_1) to predict 1 variable from another. To assess the strength of the association between variables, a nonparametric Spearman test correlation was used (95% CI), and the r_s and p_s values were calculated.

Results

From the total sample studied (106 children), 53% (56 children) had positive specific IgE results to *Phleum* major allergens but negative specific IgE results to cross-reactive pollen allergens. These patients had negative skin prick tests and negative specific IgE results to latex allergens. The other 47% (50 children) had positive specific IgE results to cross-reactive pollen allergens. From these, all were positive to latex by skin prick test, and 48 children revealed specific IgE-positive results to latex. From the 50 patients sensitized to cross-reactive grass pollen allergen (Phl p12 or Phl p 7), 48 showed sIgE to Phl p 12, 2 showed sIgE to Phl p 7, and no patients showed sIgE to Phl p 12 and Phl p 7.

Figure 1 shows the distribution profile of the specific IgE values to allergens in the group of patients ($n = 50$) who had positive results to cross-reactive pollen allergens (Phl p 7 or Phl p 12). Mean values of specific IgE to species-specific allergens of *Phleum*: Phl p 1 + Phl p 5 (71.1 kU/L) were 13 times higher than those obtained for cross-reactive *Phleum* allergens Phl p 7 (5.5 kU/L) and Phl p 12 (5.5 kU/L) and 7.5 times higher than those obtained for latex profilin (9.4 kU/L). Only 2 individuals were negative to profilin.

From these individuals ($n = 50$), 96% (48 children) had positive results to latex, peach, avocado, banana, and kiwi; 88% (44 children) to apple and pineapple; and 66% (33 children) to chestnut.

Specific IgE mean values to rubber components or derivatives were 2.1 kU/L for latex extract, 9.4 kU/L for Hev b 8 (profilin), and 0.35 kU/L or higher for Hev b 1, Hev b 5, Hev b 6, and Hev b 11.

Specific IgE mean values to fruits were 3.4 kU/L for avocado, 3.3 kU/L for peach, 1.8 kU/L for kiwi, 1.7 kU/L for pineapple, 1.6 kU/L for banana, 1.3 kU/L for apple, and 0.6 kU/L for chestnut.

Cross-reactive carbohydrate determinants gave specific IgE less than 0.35 kU/L. Mean value of specific IgE to species-specific allergens of *Plantago lanceolata*, *Olea europaea*, *Chenopodium album*, *Quercus alba*, *Cupressus sempervirens*, *Ambrosia elatior*, and *Artemisia vulgaris* were 12.9 kU/L, 2.0 kU/L, 3.4 kU/L, less than 0.35 kU/L, less than 0.35 kU/L, less than 0.35 kU/L, and 2.1 kU/L, respectively.

Figure 2 shows the linear regression analysis between the specific IgE values to Hev b 8 and the specific IgE values to Phl p 12. It also shows the analysis between the specific IgE values to Hev b 8 and the specific IgE values to latex extract in the group of patients sensitized to cross-reactive pollen allergens. The value of the correlation coefficient indicates a positive significant relationship between the variables IgE to Hev b 8 and IgE to Phl p 12: $r_1 = 0.971$, $P_1 < .0001$, and between IgE to Hev b 8 and IgE to latex extract: $r_1 = 0.933$, $P_1 < .0001$. The Spearman r value was 0.930, with $P_s < .0001$ (95% CI) for pair variables IgE to Hev b 8/IgE to Phl p 12 and 0.784 with $P_s < .0001$ (95% CI) for the pair of variables IgE to Hev b 8/IgE to latex extract.

Table 1 shows the comparison between the specific IgE values to Hev b 8 and the specific IgE values to each fruit allergenic extract assayed.

The values of the correlation coefficients for avocado ($r_1 = 0.848$; $P_1 < .0001$) and for peach ($r_1 = 0.702$; $P_1 < .0001$) indicate a positive significant relationship between the variables. A Spearman corre-

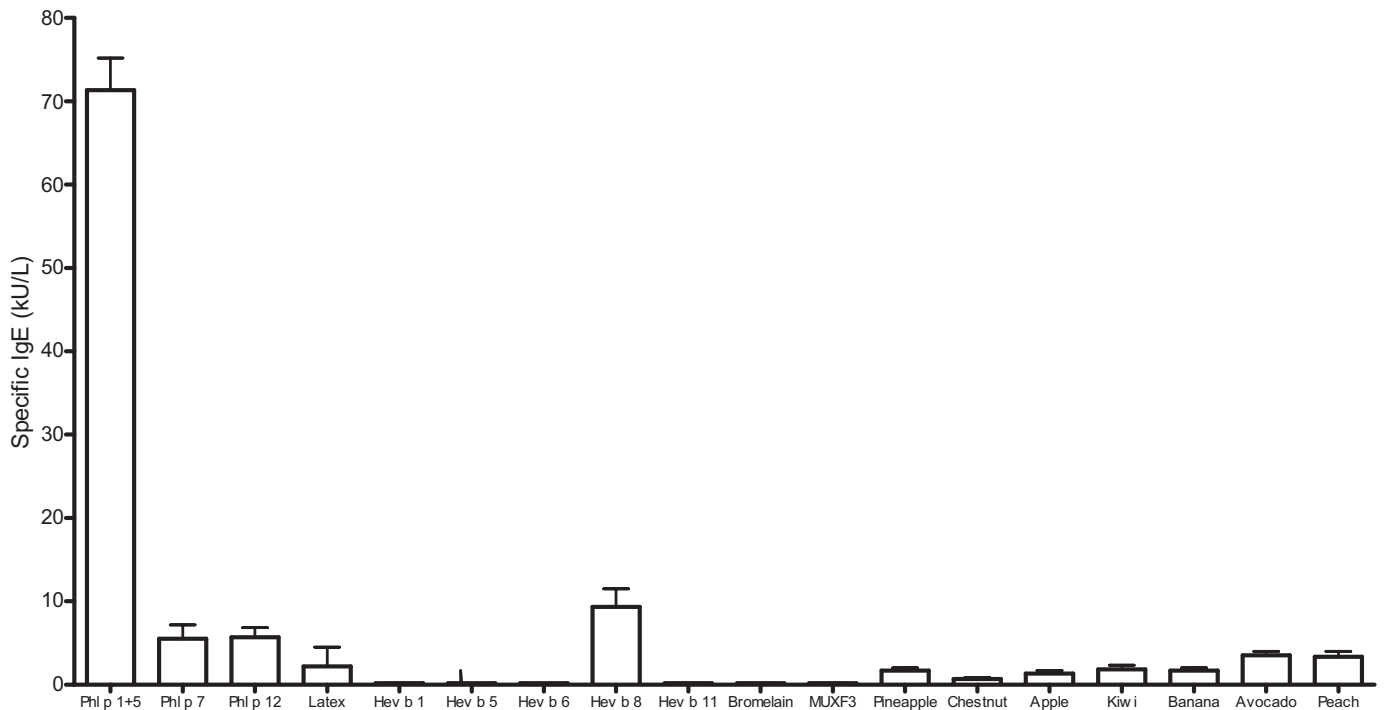


Fig. 1. Distribution profile of the specific IgE values to allergens in the group of patients ($n = 50$) who gave positive results to cross-reactive pollen allergens (Phl p 7 or Phl p 12).

lation test reinforced this relationship ($r_s = 0.830$, $P_s < .0001$ for avocado and $r_s = 0.848$, $P_1 < .0001$ for peach).

Low coefficients of correlation were demonstrated for banana and pineapple ($r_1 = 0.474$; $P_1 < .0001$), and no correlations could be demonstrated for apple ($r_1 = 0.297$; $P_1 < .0001$), kiwi ($r_1 = 0.187$; $P_1 < .0001$), and chestnut ($r_1 = 0.123$; $P_1 < .0001$).

Discussion

Although the prevalence of latex allergies in the general population seems to be low, the data published by different authors vary because of differences in the groups studied or the methodologies used to measure the allergic sensitization.

The prevalence of latex sensitization in Portuguese atopic children is 3.8% when based solely on skin prick testing, 12.1% when based singly on determination of latex-specific IgE, and 14.3% when positive results for either test are considered. Sensitivity to latex-cross-reacting foodstuffs was demonstrated in 61.5% of latex-sensitized children.² These authors comment that this study shows similar latex sensitization prevalence to that observed in other countries.

Several authors demonstrate a low prevalence of latex sensitization in the general population of both children and adults. Roberts and colleagues⁹ reported prevalence values of latex sensitization and clinical latex allergy in the general childhood population of 0.2% and less than 0.1%, respectively, and Mari et al¹⁰ reported a prevalence of latex sensitization of 0.75% for the general population and 1.2% for the atopic population. In this study, 24% of latex-sensitized patients developed symptoms after latex exposure.

Gaig et al¹¹ reported prevalence values of 5.7% and 0.2% for pollen and latex sensitization, respectively, in the Spanish general population. Taking these figures into account and making the calculation corresponding to the sample used in this work, we can estimate the prevalence of latex sensitization to be 2.6% in our region. This value is similar to that reported by Arminda and col-

leagues² and slightly higher than those found by Mari and colleagues¹⁰ and Gaig and colleagues,¹¹ all located in other countries in our immediate environment. According to these data calculated on the basis of latex sensitization through the profilin component, we estimate that a very little sensitization is caused by the species-specific latex components.

The results reported in this work demonstrate that all subjects sensitized to grass profilin were also sensitized to latex profilin. The cause of allergy in these patients and the statistic correlation between the variables IgE to grass profilin, IgE to latex profilin, and IgE to latex extract indicate that the cross-reactivity between profilins from grasses and latex is the cause of latex sensitization in these patients. Nieto and colleagues¹² suggested that profilin is a relevant allergen for patients with spina bifida and adults allergic to latex from a frequency point of view, but that it has a scarce presence in natural latex extracts and raw sources, with a subsequent low IgE induction capacity. The results here observed demonstrated that this population was not sensitized to latex, but profiling from pollens elicited a false sensitization to latex. These results discard the implication of latex but force us to take into account the profilin as possible cause of OAS from an epidemiological point of view. The evaluation of molecular allergy diagnosis together with the clinical symptoms could indicate the right role of each allergen and help to individualize the diagnosis and the prognosis of each patient. Quercia and colleagues⁹ reinforce this point, and they conclude that latex profilin is clinically irrelevant or is no longer present in latex products. The last conclusion is supported by the specific IgE measurements demonstrated in this report, in which the patients did not show clinical manifestations associated with the rubber latex, and the IgE values to Hev b 8 were 4.5 times higher than those found for IgE to latex.

The possible lack of some components in the latex material crucially reinforces the use of the molecular allergy concept be-

cause of the increase in the sensitivity and specificity of the diagnosis.

Recent publications have demonstrated that CRD can be helpful not only in defining which allergens are relevant in each case but also in predicting the severity of allergic reactions.¹³ When many single-allergen proteins are used in individual diagnosis, it is possible to check the allergenic role of each allergen and try to establish which allergens are involved in primary sensitization or cross-reactivity. It also could be possible to define some allergenic profiles and associate them with certain clinical features.⁶

Some authors, using adult patient samples, demonstrated that the CRD concept allows discrimination between latex allergies and

Table 1.

Correlation analysis between the specific IgE values to Hev b 8 and the specific IgE values to each fruit extract (allergenic source) assayed

	r_l	P_l	r_s	P_s
Hev b 8 vs peach	0.702	<.0001	0.848	<.0001
Hev b 8 vs Avocado	0.848	<.0001	0.830	<.0001
Hev b 8 vs apple	0.260	.070	0.721	<.0001
Hev b 8 vs banana	0.500	.0002	0.711	<.0001
Hev b 8 vs kiwi	0.160	.264	0.651	<.0001
Hev b 8 vs pineapple	0.452	.001	0.645	<.0001
Hev b 8 vs chestnut	0.085	.557	0.380	.0065

Abbreviations: r_l and P_l , linear-regression r and P values; r_s and P_s , nonparametric Spearman test r_s and P_s values (95% CI).

simple sensitization. In addition, they concluded that the latex profilin is clinically irrelevant in latex allergy and that it was possible to demonstrate cross-reactivity in patients with IgE to Hev b 8 and to natural rubber latex.^{6,14-16}

The data obtained in this work also support the validity of CRD as a method of choice for discriminating between latex allergy and simple sensitization.

In agreement with the previously mentioned authors, we also can conclude that latex sensitization in the children population here included is attributable to the cross-reactivity with the profilin contained in the grass pollen.

Something similar was observed with sensitization to peach and to avocado.

In the sample studied, the sensitization to grass profilin could be the cause of the sensitization to peach and to avocado and to a lesser extent to banana and pineapple.

The lack of correlation between profilin and apple, kiwi, or chestnut found here can be explained because allergens from these foods other than profilin could produce co-sensitizations.

Conversely, Raulf-Heimsoth et al¹⁶ suggest that Hev b 8 is involved in the cross-reactivity between chestnut and natural rubber latex and that Hev b 8 is a relevant allergen for the chestnut.

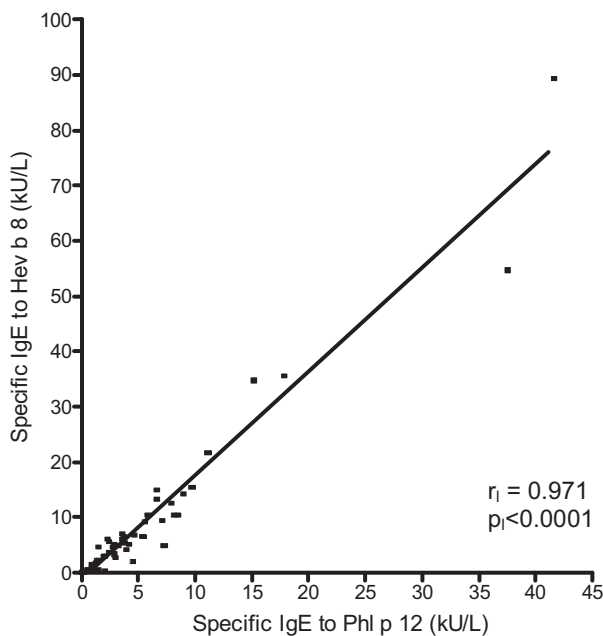
These contradictory observations could be explained because of differences in the sample populations. Although these authors include patients with clinical symptoms to latex or chestnut and with high values of specific IgE to latex and chestnut, in our study, we have included patients allergic to pollen without symptoms to latex and with very low values of specific IgE to chestnut (mean = 0.6; 33 of 50 sera showed values ≤ 0.35 kU/L).

As far as we know, no data have been reported about the role of cross-reactive profilin panallergens in the sensitization of pediatric populations. The results obtained in this work demonstrate that the apparent sensitization to latex in pediatric populations of patients allergic to grass pollen is caused by the cross-reactive profilin panallergen and is not clinically relevant.

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A)



B)

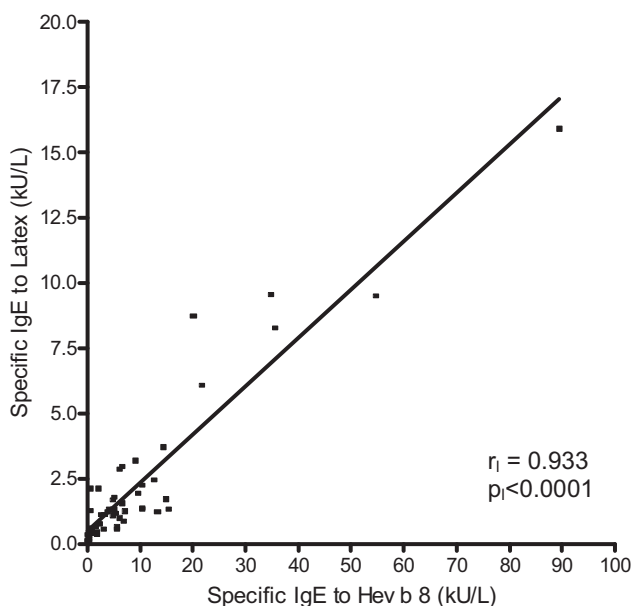


Fig. 2. Linear regression analysis. A, Correlation between specific IgE values to Hev b 8 and Phl p 12. B, Correlation between specific IgE values to Hev b 8 and latex extract.

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