

Genuine and Cross-Reactive Peanut Components in Birch-Sensitive Preschool Children

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ABSTRACT

Background: Component-resolved diagnosis (CRD), enables the identification of genuine and cross-reactive allergen molecules.

Objective: The aim of this study was to compare the results of the skin prick test (SPT), specific immunoglobulin E (sIgE), and CRD in birch-pollen-sensitive preschool children.

Methods: The subjects' mothers answered a standardized questionnaire about clinical manifestations after peanut ingestion by their children. The sIgEs were determined by the fluorescence enzyme immunoassay, and CRD by microarray technique.

Results: Children with birch-positive/peanut-positive SPT developed generalized urticaria or oral allergy syndrome within 30 minutes

after ingesting peanuts. Discordant results between peanut-SPT and peanut-sIgE, and between peanut-sIgE and peanut-genuine-CRD, respectively, were confirmed. The results of CRD distinguished genuine from cross-reactive allergens.

Conclusion: The SPT and sIgE may detect sensitization and cosensitization to allergen extracts, and CRD may detect sensitization and cosensitization to genuine and cross-reactive allergen components in preschool children. Further investigations are required to ascertain the causes of discordant results.

Keywords: allergy, birch, IgE, component-resolved diagnosis, microarray, peanut

Peanuts (*Arachis hypogaea*) belong to the plant family *Leguminosae*. Eleven peanut allergens (Ara h1 to Ara h11) have been identified.¹ Some of these allergens cause severe immunoglobulin E (IgE)-mediated allergic reactions. The most potent peanut allergens are Ara h1 (vicilin), Ara h2 (conglutin), and Ara h3 (glycinin).^{2,3} Peanut allergen Ara h8, which belongs to pathogenesis-related class 10 protein (PR-10), is a cross-reactive allergen to homologous birch allergen (Bet v1). Other peanut allergens are considered minor.⁴

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Abbreviations

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Diagnosis of peanut allergy is based on a careful history along with in vivo (skin prick test, [SPT]) and in vitro (serum peanut-specific IgE [sIgE] determination) tests. Oral food challenges (OFC), as the gold standard for food allergy diagnosis, are performed in situations when the clinical history, in vivo tests, and in vitro tests give discordant results such as negative clinical history and positive SPT and/or sIgE, or positive clinical history and negative SPT and/or sIgE. Results of SPT and allergen-specific IgE measurement to whole allergen extracts have low specificity and low positive predictive value.^{1,5} The low positive predictive value might be due to cross reactivity with profilins, PR-10, or carbohydrate determinants (CCDs).

Recently the development of component-resolved diagnosis, CRD, has made it possible to identify genuine and cross-reactive allergen molecules.¹ In particular, microarray technology offers significant progress for simultaneous determination of sIgE concentration against multiple purified natural or recombinant allergens.

The aim of the present study was to compare the results of sIgE reactivity to whole peanut extract and to peanut allergen components in preschool children with birch pollen sensitization.

Table 1. Characteristic and Outcomes of Skin Prick Test in Birch-Sensitized Children*

	Age (years)	Disease	Symptoms After Peanut Ingestion	SPT Birch (mm)	SPT Peanut (mm)	Positive sIgE	Positive CRD
Group 1							
1	6	A	U	5 x 5	5 x 5	t3, f13	Bet v1, Ara h2
2	5	A	U	4 x 4	3 x 3	t3, f13	Ara h1, Ara h2
3	1	R	OAS	2 x 3	2 x 3	t3, f13	Negative
4	5	R, A	OAS	5 x 6	4 x 5	t3	Bet v1, Ara h1, Ara h2, Ara h3
5	7	R	OAS	4 x 4	5 x 5	t3	Bet v1, Ara h8
6	4	R	U	8 x 8	10 x 10	t3, f13	Bet v1, Ara h8
7	5	R	OAS	4 x 5	2 x 3	Negative	Negative
Group 2							
1	7	A	No	3 x 3	Negative	t3	Negative
2	2	R	No	5 x 5	Negative	t3	Bet v1
3	3	R	No	3 x 4	Negative	t3	Bet v1
4	1	R	No	3 x 3	Negative	t3	Negative
5	3	R	No	3 x 3	Negative	t3	Bet v1, Ara h1, Ara h8
6	4	R, A	No	6 x 10	Negative	t3	Bet v1
7	6	R	No	3 x 5	Negative	t3	Bet v1

*Results in peanut-SPT-positive (Group 1) and peanut-SPT-negative (Group 2) children are presented.

SPT, skin prick test; sIgE, specific immunoglobulin E; CRD, component-resolved diagnosis; A, asthma; R, rhinitis; U, urticaria; OAS, oral allergy syndrome; t3, birch allergen extract, f13, peanut allergen extract; Bet v1, major birch allergen component; Ara h1, Ara h2, Ara h3, genuine peanut allergen components; Ara h8, peanut cross-reactive allergen components.

Materials and Methods

The study included birch-allergic children aged 1–6 years referred for allergy assessment during November and December 2011 (N=14). Their mothers answered a standardized questionnaire about clinical appearance of allergies in their children. All subjects experienced symptoms of allergic asthma and rhinitis. An allergic reaction after peanut consumption was considered positive if symptoms appeared within 30 minutes after consumption. Subjects were divided in two groups based on their SPT results: Group 1 – peanut-SPT-positive, N=7; Group 2 – peanut-SPT-negative, N=7).

Clinical evaluation included history, physical examination, SPT, determination of sIgE, and allergen CRD. Sensitization was tested by SPT using Alyostal allergen extracts (Stallergenes, France), and a wheal diameter of 3 mm or larger was considered positive. Concentrations of sIgEs were determined by fluorescence enzyme immunoassay (ImmunoCAP, Phadia, Uppsala, Sweden). Sensitization profiles of birch major allergen Bet v1, genuine peanut allergens (Ara h1, Ara h2, Ara h3), and cross-reactive al-

lergens in peanut (Ara h8) were performed using the microarray technique ImmunoCAP ISAC (Phadia, Uppsala, Sweden).

Statistical concordance between SPT, sIgE, and CRD was tested using interrater agreement. Agreement was quantified by kappa (κ) coefficient of agreement with κ values from 0.81 to 1.00 indicating very good concordance; 0.61 to 0.80, good concordance; 0.41 to 0.60, moderate concordance; 0.21 to 0.40, fair concordance; and 0.20, poor concordance. $P < 0.05$ was considered statistically significant.⁶

Results

In Group 1, two children developed asthma, four children developed rhinitis, and one child developed both (**Table 1**). After peanut ingestion, three patients developed generalized urticaria and four developed oral allergy syndrome (OAS). In Group 2, one child developed asthma, five developed rhinitis, and one child had symptoms of both. In one patient (No. 6) from Group 1, mean wheal

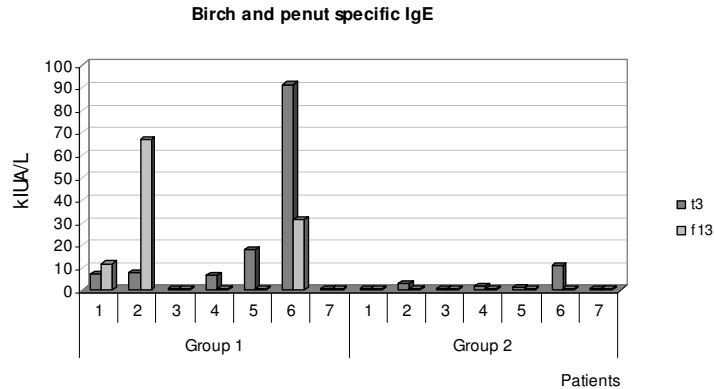


Figure 1 Concentration of birch (t3) and peanut (f13) specific IgE in birch-positive/peanut-SPT-positive (Group 1) and in birch-positive/peanut-SPT-negative (Group 2) children. t3, birch allergen extract; f13, peanut allergen extract

diameter for SPT with birch allergen extract was 8x8 mm, and for peanut allergen extract was 10x10 mm; in others, it ranged from 3 to 5 mm for both allergens. In Group 2, the mean wheal diameter for SPT with birch allergen extract was 6x10 mm, in the other 6 children it ranged from 3 to 5 mm.

Specific IgEs (**Figure 1**) to birch extract (t3) were increased in all of the Group 1 subjects (range: 0.54 kIU_A/L in patient No. 7 to 91.3 kIU_A/L in patient No. 6). Specific IgEs to peanut extract were below 0.35 kIU_A/L in three Group 1 subjects (Nos. 4, 5 and 7); in other Group 1 subjects, values were 11.7 kIU_A/L (No. 1), 66.9 kIU_A/L (No. 2), 0.8 kIU_A/L (No. 3) and 31.2 kIU_A/L (No. 6). In Group 2, increased sIgE to birch extract was confirmed in subjects 1 (0.41 kIU_A/L), 2 (2.86 kIU_A/L), 3 (0.60 kIU_A/L), 4 (1.60 kIU_A/L), 5 (0.90 kIU_A/L) and 6 (10.62 kIU_A/L); sIgE was not detectable (<0.35 kIU_A/L) in subject 7. Specific IgEs to peanut extract were below 0.35 kIU_A/L in all Group 2 children.

In Group 1, CRD results confirmed that four children had positive Bet v1 (Nos. 1, 4, 5, 6), three children (3/7) had positive genuine Ara h (Nos. 1, 2, 4), three children had positive genuine Ara h (subjects 2, 11, 30), and two children had positive cross-reactive Ara h8 (subjects 5 and 6). In Group 2, Bet v1 increased in five subjects (2, 3, 5, 6, 7), and genuine Ara h1 was confirmed in patient 5 (0.5, ISAC Standardized Units, [ISU]).

In Group 1, birch SPT/sIgE positive concordance was found in 6 of 7 subjects; and SPT-negative/sIgE-negative results were not observed. One child had SPT/sIgE-discordant (SPT-/sIgE+) results. In Group 2, birch SPT-positive/sIgE-positive concordance was observed in all children.

In Group 1, there were four children with birch sIgE-positive/CRD-positive results (subjects 1, 4, 5, 6), one

child with birch sIgE-negative/CRD-negative results (No. 7), and two children (Nos. 2, 3) with birch sIgE/CRD-discordant results. Peanut sIgE-positive/CRD-genuine peanut positive results correlated in two patients (Nos. 1, 2), peanut sIgE negative/CRD-genuine peanut negative concordant results were observed in three patients (Nos. 5, 6, 7), and peanut sIgE positive/CRD-genuine peanut negative, and peanut sIgE-negative/CRD-genuine peanut positive discordance was confirmed in patients 3 and 4, respectively.

In Group 2, there were five children with birch sIgE-positive/CRD-positive results (subjects 2, 3, 5, 6, 7), and two children with birch sIgE-positive/CRD-negative results (1 and 4). Peanut sIgE-positive/CRD-positive results were not observed, but peanut-sIgE-negative/CRD genuine-positive results were observed in one subject (No. 5). Cross-reactive Ara h 8 was positive in patient No. 5.

Discordance between clinical manifestation after peanut consumption and CRD was found in Group 1, since genuine peanut allergen components were not detected in 4/7 children (**Table 1**). In Group 2, one child was positive for Ara h1 (subject 5), although that child was not symptomatic after peanut consumption.

Moderate overall concordance ($\kappa = 0.429$) between peanut SPT and sIgE was found. Poor concordance between birch SPT and sIgE ($\kappa = 0$), between birch-sIgE and CRD (Bet v1) ($\kappa = -0.135$), and between sIgE and peanut-genuine CRD (Ara h 1-3) ($\kappa = 0.186$) was observed.

Birch and peanut allergen profiles of birch-sensitive children were determined based on CRD (**Table 1**, **Figure 2**). In Group 1 (**Table 1**, **Figure 2A**), peanut sensitivity was confirmed in patient 2, birch and peanut cosensitization was confirmed in patients 1 and 4, and

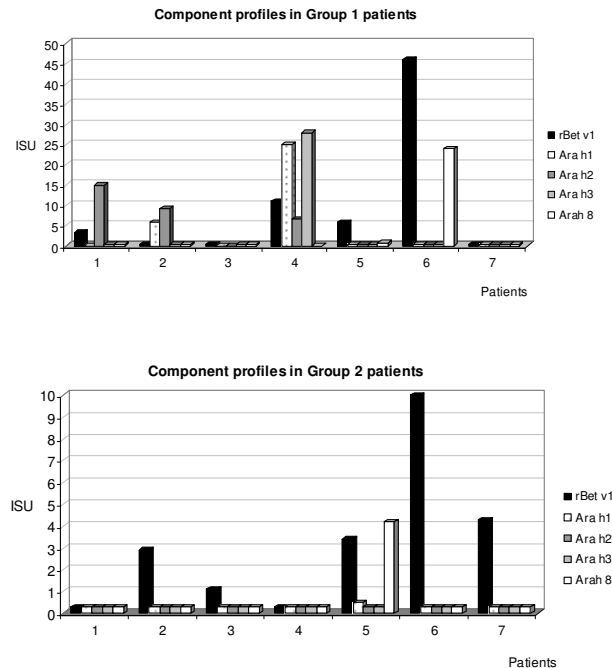


Figure 2 birch- and peanut-component profiles: A- in birch-positive/peanut-SPT-positive (Group 1) and B - in birch-positive/peanut-SPT-negative (B, Group 2) children. ISU, ISAC standardized units; Bet v1 – major birch allergen component; Ara h1, Ara h2, Ara h3 – genuine peanut allergen components; Ara h8 – peanut cross-reactive allergen components

birch-peanut cross reactivity was confirmed in patients 5 and 6. Hypersensitivity was not confirmed in patients 3 and 7. In Group 2 (Table 1, Figure 2B), birch sensitivity was confirmed in patients 2, 3, 6, and 7; birch-peanut cosensitization was confirmed in patient No. 5, and hypersensitivity was not confirmed in patients 1 and 4.

Discussion

CRD may improve conventional allergy diagnostics by distinguishing between genuine and cross-reactive allergens in susceptible individuals. Neither SPT nor sIgE detects cross-reactive allergens; CRD may be a useful modification of conventional diagnostic procedures in preschool children sensitive to cross-reactive Ara h8.

Birch- and peanut-allergen extracts used in SPT and specific IgE tests contain a mix of allergenic and nonallergenic proteins, and therefore cannot distinguish between sensitivity to genuine and cross-reactive components. These two tests, (SPT and sIgE) may be used as screening tests for peanut allergy.⁷ Reports in the literature suggest that thresholds positive for peanut-SPT and peanut-sIgE are different from other food or inhalant allergens. Wheal diameter of at least 7 mm in peanut-sensitive children has been reported to have a sensitivity of 83%, specificity of 97%, and a positive predictive value of 93%.⁸ In the present study, the patient who developed

urticaria after peanut ingestion with wheal diameter of 10 mm and had a concentration of peanut sIgE of 31.2 kIU_A/L, the concentration of cross-reactive Ara h8 IgE also was significantly increased (24 ISU). Moreover, genuine peanut allergen components Ara h1, Ara h2, and Ara h3 in that subject were not detected. In two other subjects who developed urticaria, wheal diameters were less than 6 mm.

Johannsen et al, suggested that wheal diameter below 7 mm and peanut-specific IgE below 2 kIU_A/L may identify preschool children with tolerance to peanuts.⁹ One child in our study who developed OAS after peanut ingestion with positive SPT and undetectable peanut sIgE, was positive for genuine peanut allergens Ara h1 – 25 ISU, Ara h2 – 6.6 ISU, and Ara h3 – 28 ISU. We suspect that the sIgE result in that patient was falsely negative. According to the results of Sampson et al, peanut sIgE greater than 15 kIU_A/L predicts clinical symptoms.¹⁰ Rancé et al state that there is no need to perform an oral challenge test in subjects with wheal diameter at least 16 mm and sIgE at least 57 kIU_A/L.⁷ Nicolaou et al have shown that the peanut component Ara h2 was the most important predictor of clinical allergy.¹¹ About 50% of preschool children with no history of peanut ingestion and positive SPT to peanut allergen can tolerate peanuts.⁹

Moderate overall concordance ($\kappa = 0.429$) between peanut SPT and sIgE and poor concordance ($\kappa = 0.186$) between peanut sIgE and peanut-genuine CRD reveal

that direct comparison between SPT, sIgE, and CRD is still unsatisfactory. The causes of discordant results may be clinical (skin reactivity in small children, different allergen-extract sources for in vivo and in vitro tests, season of testing, geographical region) and analytical (immunoassay design, different allergen-extract sources in various immunoassays, diverse specificities of the antibodies and binding proteins in different immunoassays, low concentration of allergen molecules in crude extracts, interferences in immunoassays).^{4,12}

Limitations of the present study include a small number of subjects, incomplete panels in CRD, and the lack of comparison with oral peanut challenge.

The results of our study demonstrate that CRD may differentiate sensitization to genuine peanut components from sensitization to cross-reactive allergens in birch-sensitive preschool children. In addition, this study has shown that the approach has to be individualized for each patient. Further investigations are required to explain the discordant results we observed. **LM**

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