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Bet v 1-specific IgE levels and PR-10 reactivity discriminate silent sensitization from phenotypes of birch allergy

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To the Editor

For certain forms of allergy (eg, food allergy, respiratory allergy, skin allergy), molecular allergy diagnosis can provide clinically useful information for the prediction of symptoms. These predictive algorithms are based on determination of specific IgE levels to defined allergen molecules as well as on IgE reactivity profiles. Such information can be established in a single step by multiplex technologies like allergen microarrays. For example, levels of IgE to certain food and respiratory allergens were found to be associated with severity of clinical phenotypes (eg, anaphylaxis, asthma).^{1,2} Similar observations were made regarding the occurrence of more severe respiratory symptoms in polysensitized subjects.³ Regarding allergy to birch pollen which affects more than 100 million patients worldwide, mainly in Northern Europe and Central Europe, certain parts of North America, Australia, and Japan,⁴ the association between IgE sensitization to allergen molecules and clinical phenotypes is less clear. However, the major birch pollen allergen, that is, Bet v 1, and the cross-reactive allergens in pollen- and plant-derived food responsible for the birch pollen-associated oral allergy syndrome have been well characterized.⁵ In the Swedish BAMSE birth cohort, the

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Conflicts of Interest

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evolution of IgE reactivities to micro-arrayed Bet v 1 and Bet v 1-related PR-10 proteins was investigated recently. In this study, it was demonstrated that Bet v 1-specific IgE levels measured early in life can be used to calculate the likelihood if children are prone to develop respiratory symptoms to birch later in life.⁶ However, data are controversial if specific IgE levels to Bet v 1 and Bet v 1-related PR-10 allergens are associated with certain symptoms of birch pollen-related allergy. One study indicates that Bet v 1-specific IgE levels may predict oral allergy syndrome and another one suggests Mal d 1-specific IgE as biomarker,^{7,8} whereas another study did not find such an association, neither for Bet v 1 nor for Mal d 1.⁹ There is also no evidence that certain IgE reactivity profiles to Bet v 1 and Bet v 1-related PR-10 allergens are associated with respiratory or skin manifestations of birch pollen-associated allergy and certain clinical phenotypes.

The aim of the present study was to investigate whether IgE reactivity profiles and/or threshold levels of IgE to Bet v 1 and Bet v 1-related PR-10 allergens can be determined that allow to differentiate between silent IgE sensitization, certain phenotypes of birch pollen-associated allergy, and/or multimorbidity. Children from Moscow region (Russia), a highly birch pollen-exposed area, were screened by using a validated ISAAC questionnaire for birch pollen allergy (Figure S1 and Methods, Appendix S1). This approach is frequently used for preliminary clinical characterization of trial participants (see references S1 and S2 of the Appendix S1) and allowed us to establish one group of children with birch pollen-related allergy symptoms (group 1: $n = 103$) and a second group devoid of allergic symptoms (group 2: $n = 97$; Table 1). There was no significant difference between the two groups regarding age (range: 10-16 years) and gender distribution (Table 1). Following questionnaire-based screening, children were subjected to detailed clinical assessment of their allergy symptoms (Methods, Appendix S1) the result of which are displayed in Table 1 (allergic rhinoconjunctivitis: AR, $n = 65$; asthma: AS, $n = 28$; oral allergy syndrome: OAS, $n = 34$; atopic dermatitis: AD, $n = 34$). Using the MeDALL allergen microarray (Methods, Appendix S1), we then tested the sera from the symptomatic (group 1) and from the asymptomatic children (group 2) for IgE reactivity to Bet v 1 and a panel of eight additional PR-10 allergens from alder pollen, hazelnut, apple, peanut, peach, celery, kiwi and soy (respiratory PR-10 allergen: Aln g 1; PR-10 food allergens: Cor a 1.0401 Mal d 1, Ara h 8, Pru p 1, Api g 1, Act d 8, Gly m 4) that are cross-reactive to Bet v 1 (Figure 1A,B). Interestingly, Bet v 1-sensitized children were not only found in the symptomatic group ($n = 65$ out of 103), but almost one third of the asymptomatic children also showed Bet v 1-specific IgE reactivity ($n = 30$ out of 97) (Table S2; Figure 1A,B). In line with previous studies, IgE sensitization was dominated by Bet v 1, followed by less frequent sensitizations to respiratory and food PR-10 allergens (Table S2; Figure 1A,B). Median IgE levels to Bet v 1 and Bet v 1-related PR-10 allergens were significantly ($P < 0.0001$) lower in the asymptomatic children (1.12 ISU) as compared to subjects with birch pollen-related allergic symptoms (19.07 ISU; Table S2 and Figure 1). Using a cutoff level of 6.8 ISU (Figure S2), children without symptoms could be discriminated from children with any type of birch pollen-related symptoms with an odds ratio (OR) of 14.1 (Table 1). As for Bet v 1 (Figure 1D), specific IgE levels to the other Bet v 1-related PR-10 allergens were also significantly lower in the asymptomatic children compared to children with different birch pollen-related symptoms (Figure S3). Interestingly, children with birch pollen-related oral allergy

syndrome (OAS) had significantly higher Bet v 1-specific IgE levels than children with birch pollen-related rhinoconjunctivitis, asthma, or atopic dermatitis (Figure 1D; $P < 0.0001$). Children with OAS also had higher IgE levels to Bet v 1-related respiratory PR-10 allergens (ie, Aln g 1; Figure S3A) as well as to Bet v 1-related food allergens (ie, Cor a 1.0401, Mal d 1, Ara h 1, Pru p 1, Api g 1, Act d 8, Gly m 4; Figure S3B). In fact, children with Bet v 1-specific IgE > 6.8 ISU had an OR of 18.3 to suffer from OAS (Table 1). This value was much higher than for any other isolated birch pollen-associated symptom or any combination of symptoms (ie, rhinitis, asthma, atopic dermatitis; Figure S2B,C,E; Table 1). Diagnostic sensitivity and specificity of the defined threshold levels of Bet v 1-specific IgE are indicated in Table 1.

Bet v 1-sensitized children of group 1 reacted most frequently with the major respiratory PR-10 allergen from alder (Aln g 1:96.9%), followed by the most frequently recognized PR-10 food allergens, that is, Cor a 1.0401 from hazelnut (87.6%) and the major apple allergen Mal d 1 (87.6%), whereas $<80\%$ of the children showed IgE reactivity to the other PR-10 food allergens (Table S3). Interestingly, the hierarchy of cross-reactivity did not fully match the sequence identities because the major allergen from peach, Pru p 1, showed higher sequence identity to Bet v 1 (ie, 59%) than the major apple allergen Mal d 1 (ie, 56%) but was only recognized by 72% of Bet v 1-allergic patients (Tables S3, S4). The latter may indicate that the higher IgE recognition frequency of Mal d 1 could be not only due to structural similarities of the allergens but to more frequent exposure due to higher consumption of apple compared to peach.

We finally analyzed our data for a possible relation between the numbers of recognized PR-10 allergens and allergic multimorbidity. Indeed, we found that patients suffering from 3 to 4 types of allergic symptoms more frequently exhibited broader IgE reactivity, that is, to seven or eight of the PR-10 allergens in addition to Bet v 1 (Figure S4). This was also corroborated by showing a moderate, yet significant association between the sum of IgE levels to the different PR-10 allergens on the microarray with the number of comorbidities (Figure S5; Spearman's $\rho = 0.37$, $P < 0.01$). This indicates that multimorbidity in birch pollen allergy may be related to the number of recognized PR-10 allergens from different allergen sources.

Our study thus demonstrates that it is possible to determine serological cutoff levels for Bet v 1-specific IgE, which discriminate children without symptoms from symptomatic children. Interestingly, Bet v 1-specific IgE levels were significantly higher in patients with OAS than with birch pollen-related respiratory and skin symptoms. Even patients with asthma and atopic dermatitis had significantly lower Bet v 1-specific IgE levels than patients with OAS. Due to almost identical methodologies, we assume that results obtained with the MedALL chip can be directly extrapolated to ImmunoCAP ISAC (Phadia AB, Thermo Fisher), a commercially available allergen microarray. Furthermore, we found that allergic multimorbidity was associated with sensitization to a broader panel of PR-10 allergens in addition to Bet v 1, an observation that is in line with results obtained for different types of allergen families such as LTP.¹⁰ Finally, our results show an unexpectedly high frequency of clinically silent IgE sensitization to the major birch pollen allergen Bet v 1 and Bet v 1-related PR-10 allergens in children exposed to birch pollen. Therefore, determination of

specific IgE levels to Bet v 1 and PR-10 allergens by microarray might be clinically useful as a potential biomarker for the discrimination between silent and symptomatic sensitization and as a surrogate marker for the severity of the clinical phenotype in birch pollen-associated allergy. As this technology consumes only low amounts of serum samples, it is well suited for allergy diagnosis in pediatric patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Numbers (y-axes) of (A) allergic (n = 103) and (B) nonallergic subjects (n = 97) with IgE-reactivity to Bet v 1 from birch and to Bet v 1-related PR-10 allergens (x-axes): Aln g 1, Mal d 1, Cor a 1.0401, Ara h 8, Pru p 1, Api g 1, Act d 8, Gly m 4. Ranges of allergen-specific IgE levels are color-coded. C and D, Bet v 1-specific IgE levels (y-axes; ISU) in Bet v 1-positive subjects shown for any type of allergic symptoms (C) or for individual manifestations of birch pollen allergy (D), that is, allergic rhinitis (AR: n = 65), asthma (AS: n = 28), oral allergy syndrome (OAS: n = 34), and atopic dermatitis (AD: n = 34), in comparison with sensitized but asymptomatic subjects (N-A: n = 30). Box plots display upper and lower quartiles with medians (horizontal lines), minima, and maxima. Mean

values are indicated by an x within the boxes. Outliers ($>1.5x$ the interquartile range from the median) are represented by circles. Significant differences are indicated: $P < 0.0001$

Table 1
Odds ratios (OR, 95% CI) for different types of birch-related symptoms in Bet v 1-
sensitized subjects (n = 95) with IgE to Bet v 1 above the respective cutoff values

Type of Symptoms	symptoms Yes/No	n	Bet v 1-spec. IgE			OR	95% CI	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
			cutoff (ISU)	n, below cutoff	n, above cutoff				
All types (AR, AS, AD, OAS)	Yes	65	6.8	17	48	14.1	4.6-42.6	73.8 (62.1-83)	83.3 (66.4-92.7)
	No	30		25	5				
AR	Yes	65	1.3	14	51	5.4	2.1-13.9	78.5 (67-86.7)	60 (40.8-76.8)
	No	30		18	12				
OAS	Yes	34	6.8	3	31	18.3	5.0-66.9	91.2 (77-97)	63.9 (51.4-74.8)
	No	61		39	22				

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; AS, allergic asthma; BAMSE cohort, Barn Allergi Miljö Stockholm Epidemiologi cohort; IgE, immunoglobulin E; ISAC, immuno solid-phase allergen chip; ISU, ISAC Standardized Units; MeDALL, mechanisms of the development of allergy; OAS, oral allergy syndrome; PR-10 allergen, pathogenesis-related allergen; r, recombinant.