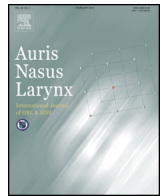




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Clinical benefit of component-resolved diagnosis in Japanese birch-allergic patients with a convincing history of apple or peach allergy

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ABSTRACT

Objective: In northern Japan, birch pollen is the major allergen in pollinosis, while oral allergy syndrome (OAS) is caused primarily by apple and peach, and is almost exclusively related to birch pollinosis. To clarify the clinical benefit of allergen-based component-resolved diagnosis (CRD) in Japanese birch-allergic patients with OAS, we present an analysis of IgE profiles in response to crude extracts and recombinant component-resolved allergen to birch pollen and Rosaceae fruits allergens.

Methods: The sera of 30 patients with birch pollen-related OAS to apple or peach were analyzed for specific IgE reactivity to pathogenesis-related class 10 (PR-10) family (birch: rBet v 1, apple: rMal d 1, and peach: rPru p 1), profilin (birch: rBet v 2 and peach: rPru p 4), and lipid transfer protein (LTP) (apple: rMal d 3 and peach: rPru p 3) allergens, as well as to conventional crude, unfractionated extracts (birch: T3, apple: f49, and peach: f95) using the ImmunoCAP SystemTM. Allergen-specific IgE values <0.35 kUA/L were considered negative.

Results: Of the 30 sera CAP-positive for natural birch pollen extract, 28 (93.3%) exhibited specific IgE against Bet v 1, and two (6.7%) contained specific IgE against Bet v 2. Of the 26 sera of OAS to apple patients, only 17 were positive for specific IgE against f49 extract (65.4%); however, 24 were positive for specific IgE against rMal d 1 (92.3%). Similarly, only 17 of the 23 sera of OAS to peach patients contained specific IgE against the f95 extract (73.9%); however, 22 were positive for specific IgE against rPru p 1 (95.7%).

Conclusion: Our data suggest that CRD constitutes a reliable tool for the diagnosis of birch pollen-related OAS.

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1. Introduction

Diagnosis of IgE-mediated food allergy is typically made using clinical history in combination with skin prick testing (SPT) and/or immunoassays of serum food-specific IgE levels. Double-blind placebo-controlled oral food challenge (OFC) is currently the gold standard test for diagnosing food allergy. However, OFC may cause the patients severe immediate local or

systematic allergic reactions such as laryngeal edema and anaphylaxis. Allergen component-resolved diagnosis (CRD) is a new methodology used in clinical food allergy diagnosis. Instead of relying on the crude allergen extracts used in standard allergic diagnostics, CRD utilizes purified or recombinant allergens to identify the specific molecules that cause sensitization or allergy [1].

It is widely accepted that CRD using recombinant allergens is a potentially superior tool for IgE-based diagnostic tests compared to tests using extracts from natural source materials [2]. CRD can identify cross-reactivity to specific components that are similar to allergens from different pollen species or foods [3]. In northern Japan, birch pollen is the major pollinosis allergen, while pollen-associated food allergy is caused primarily by apple and peach, and is related almost exclusively to birch pollen allergy [4]. However, to date, the clinical benefits of component-resolved diagnosis in birch-allergic patients with oral allergy syndrome (OAS) against these Rosaceae fruits are yet to be examined. In this study, we present an analysis of IgE profiles in response to crude, unfractionated extracts and recombinant component-resolved allergen to birch pollen and Rosaceae fruits allergens using an immunoenzymatic CAP method.

2. Methods

This study used the sera of 30 patients with birch pollen-related oral allergy symptoms to apple or peach with specific IgE against birch pollen extract >0.70 kUA/L and the sera of 10 healthy control subjects without any symptoms of OAS. All patients complained of oral allergy symptoms such as repetitive pruritus and/or angioedema of the lips, tongue, throat, and/or palate due to the consumption of raw apples or peaches within 1 year. All patients were recruited from the outpatients of the department of Otolaryngology at Sapporo Medical University or the Yamamoto ENT clinic in the period between April and July 2015. Informed consent was obtained from all patients, and the study was approved by the ethics committee of Sapporo Medical University. Demographic and clinical characteristics

of the patients and controls are summarized in Table 1. 26 patients had OAS to apple and 23 patients had OAS to peach.

IgE-specific antibody levels against conventional crude, unfractionated extracts (birch: T3, apple: f49, and peach: f95), recombinant pathogenesis-related class 10 (PR-10) family (birch: rBet v1, apple: rMal d1, and peach: rPru p1), profilin (birch: rBet v2 and peach: rPru p4), and lipid transfer protein (LTP) (apple: rMal d3 and peach: rPru p3) allergens were evaluated using the ImmunoCAP method (Thermo Fisher Scientific, Uppsala, Sweden). The cutoff value for the assay was set at 0.35 kUA/L. The paired *t* test was used to analyze the differences between IgE-specific antibody levels against conventional crude, unfractionated extracts (apple: f49 and peach: f95) and recombinant PR-10 family (apple: rMal d1 and peach: rPru p1).

Spearman correlation coefficients were used to evaluate whether the IgE serum levels for rBet v1, crude allergens, and PR-10 family allergens were correlated.

3. Results

Of the 30 sera demonstrating CAP-positive results for natural birch pollen extract, 28 (93.3%) contained specific IgE against rBet v1, and four (13%) exhibited specific IgE against rBet v2 (Table 2). Of these four sera with specific IgE against rBet v2, three sera contained specific IgE against profilin of peach (rPru p4). LTP (rMal d3 and rPru p3) reactivity was documented in only one subject (3%).

As shown in Fig. 1, there was a strong correlation between the IgE serum levels against natural birch pollen extract and against recombinant Bet v1 ($r = 0.99$, $p = 7.0 \times 10^{-26}$). Only 17 of 26 sera of OAS to apple patients had specific IgE against the f49 extract (65.4%; Fig. 2A), while 24 cases were positive for specific IgE against rMal d1 (92.3%; Fig. 2B). The levels of rMal d1-specific IgE were significantly ($p = 0.0016$) higher compared with IgE levels of f49 extract. Similarly, only 17 of 23 sera of OAS to peach patients contained specific IgE against the f95 extract (73.9%; Fig. 3A), while 22 were positive for specific IgE against rPru p1 (95.7%; Fig. 3B). The levels of

Table 1
Demographic characteristics of birch-allergic patients and control subjects.

Type of OAS	Only apple (<i>n</i> = 7)	Both apple and peach (<i>n</i> = 19)	Only peach (<i>n</i> = 4)	Total (<i>n</i> = 30)	Controls (<i>n</i> = 10)
Sex (male/female)	3/4	6/13	1/3	10/20	6/4
Age	35 (29–67)	44 (18–79)	29 (23–32)	36.5 (18–79)	36 (26–45)
Specific IgE to birch pollen (T3) (kUA/L)	23.1 (1.2–100)	15.9 (5.3–100)	10.4 (1.3–39)	16.2 (1.2–100)	<0.35

Data expressed as median values and range (in brackets).

Table 2
Percentage of birch-allergic patients with IgE reactivity to the recombinant allergens Bet v1 and Bet v2.

Recombinant allergens	Type of OAS % (<i>n</i>)						Controls (<i>n</i> = 10)
	Only apple (a) (<i>n</i> = 7)	Both apple and peach (b) (<i>n</i> = 19)	Only peach (c) (<i>n</i> = 4)	Apple (a + b) (<i>n</i> = 26)	Peach (b + c) (<i>n</i> = 23)	Total (a + b + c) (<i>n</i> = 30)	
Bet v1	86 (6)	100 (19)	75 (3)	96 (25)	96 (22)	93 (28)	0 (0)
Bet v2	43 (3)	5 (1)	0 (0)	15 (4)	4 (1)	13 (4)	0 (0)

Data expressed as positive % (*n*).

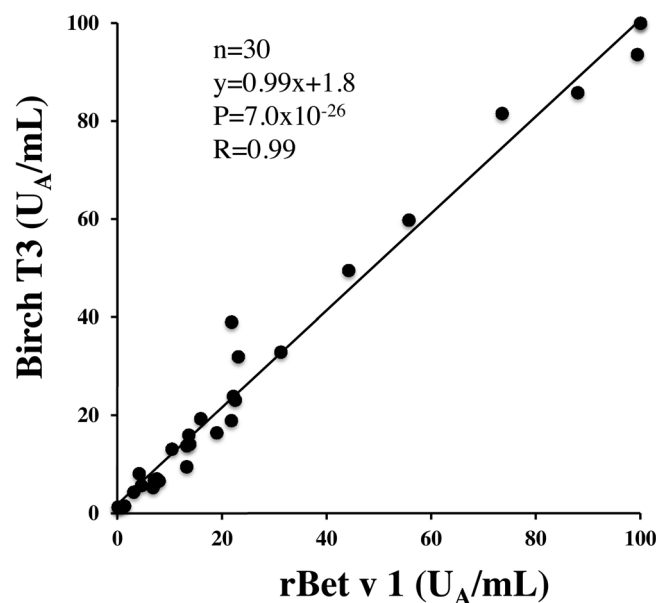


Fig. 1. Significant correlation between the specific IgE levels against recombinant Bet v 1 and birch pollen extract (T3).

rPru p 1-specific IgE were significantly ($p = 0.0038$) higher compared with IgE levels of f95 extract.

Linear regression analysis was used to determine the relationship between the serum IgE levels against rBet v 1, crude food allergens and PR-10 family allergens. The regression line slopes for the serum IgE levels against the crude apple (Fig. 4A) and peach (Fig. 5A) allergen versus the rBet v 1 serum IgE levels were very low (apple crude allergen: 0.17 and peach crude allergen: 0.12). In contrast, the regression line slopes for the serum IgE levels against the PR-10 related allergen components (rMal d 1: Fig. 4B and rPru p 1: Fig. 5B) versus the rBet v 1 serum IgE levels were relatively steep (rMal d 1: 0.54 and rPru p 1: 0.51).

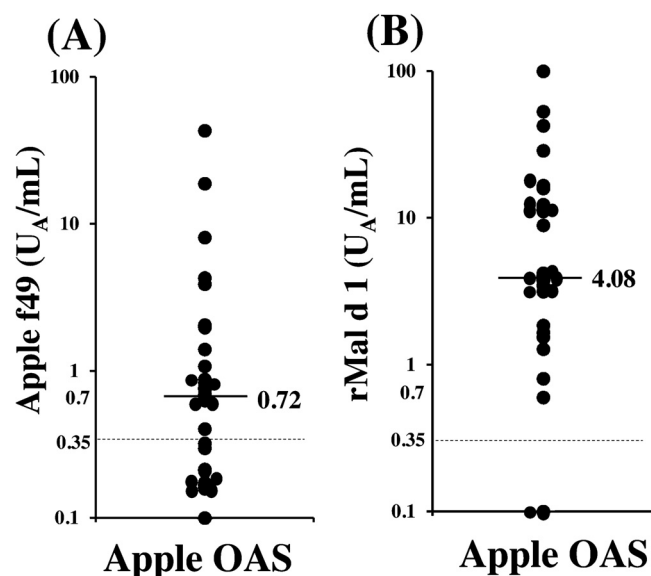


Fig. 2. Specific IgE recognition of the unfractionated apple extract (A) and the recombinant PR-10 allergen (B), determined in sera from 26 patients with OAS against apple. Median values are indicated.

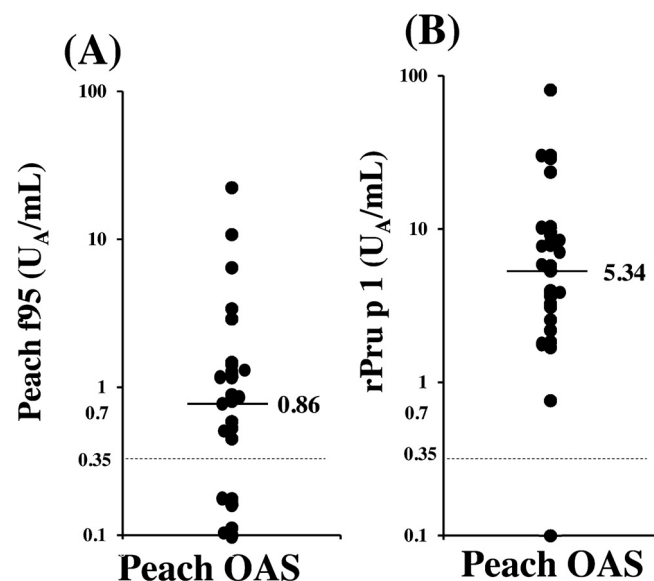


Fig. 3. Specific IgE recognition of the unfractionated peach extract (A) and the recombinant PR-10 allergen (B), determined in sera from 23 patients with OAS against peach. Median values are indicated.

4. Discussion

In this study, we analyzed the IgE-reactivity profile in birch pollen-allergic patients with OAS from northern Japan. We have previously reported the prevalence of sensitization to rBet v 1 and Bet v 2 were in birch-allergic patients, demonstrating there was no significant difference in IgE positivity to these birch allergens between patients with OAS and patients without OAS [4]. A high correlation was apparent between the serum IgE levels against natural birch pollen extract and the IgE levels against recombinant Bet v 1 similar to the findings of our previous report [4]. While IgE antibodies to Bet v 1 are highly prevalent in patients from Sweden, Finland and Austria (≥ 98) and common in French patients (90%), less than 70% of the patients from Switzerland and northern Italy exhibited these antibodies [5]. In contrast, IgE reactivity to Bet v 2 is rare among Scandinavian patients, and was more prevalent in the patients living in central or southern Europe [5,6]. Also, IgE reactivity to LTP (rMal d 3 and rPru p 3) is rare among birch pollen-allergic patients from northern Japan. Thus, the data in this study suggest that the specificity of the birch pollen allergen-related IgE reactivity profiles in Japanese birch-allergic patients correspond to that exhibited by Scandinavian patients, which differs from the results obtained in patients from northern Italy.

Our data suggest that quantification of serum IgE antibodies against the recombinant major apple (rMal d 1) and peach (rPru p 1) allergens using ImmunoCAP constitutes reliable tools for the diagnosis of OAS to apple and peach in birch pollen allergy patients and that this method is superior to the traditional ImmunoCAP method utilizing crude allergen extracts (Figs. 2 and 3). PR-10 family allergens are intracellular pathogenesis-related (PR) proteins with a molecular mass of approximately 17–18 kDa [7]. A high degree of structural homology has been demonstrated between the birch rBet v 1, apple rMal d 1 [8] and peach rPru p 1 [9], PR-10 family

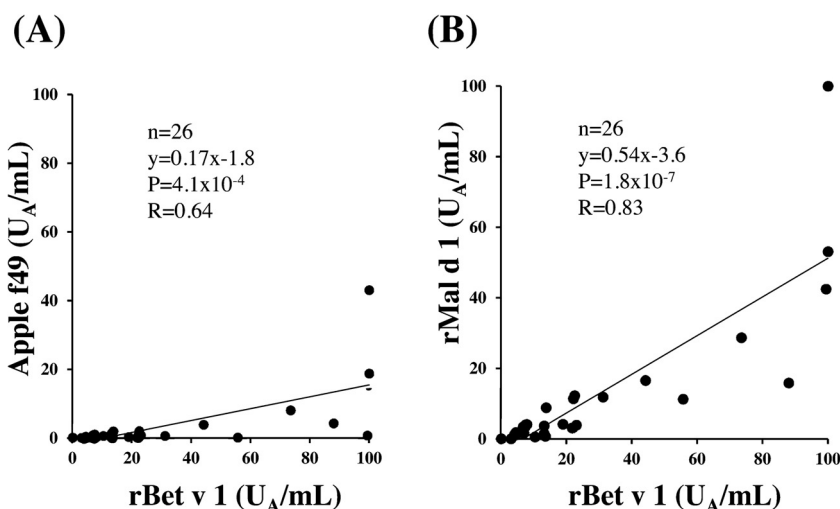


Fig. 4. Linear regression analysis of IgE values against rBet v 1 and apple-specific IgE values. (A) Unfractionated apple extract (f49) versus rBet v 1; the slope of the regression line, 0.17. (B) Recombinant PR-10 allergen (rMal d 1) versus rBet v 1; the slope of the regression line, 0.54.

allergens. The advantages of allergen CRD demonstrated in this study might be owing to the steep regression line slopes for the serum IgE levels against PR-10-related allergen components versus the serum IgE levels against rBet v 1 as shown in Figs. 4 and 5. CRD has garnered a lot of attention in recent years in the diagnosis of food allergies since the method enables a more accurate assessment while requiring less patient serum. CRD uses purified allergen proteins from natural allergen sources or recombinant proteins expressed from allergen-encoding complementary DNA instead of crude allergen extracts consisting of a mixture of allergenic and non-allergenic components [1]. Recently, a number of studies have demonstrated that CRD may improve the specificity of allergy testing for a variety of foods including peanut, milk, and egg [2,3]. Ebo et al. reported that CRD performed using microarray or ImmunoCAP is a reliable tool for the diagnosis of apple-mediated OAS in birch pollen allergy based on the analysis of the sera of 20 patients with OAS in Belgium [10].

In addition, a previous study has shown that CRD improved the detection of soy sensitization profiles, further demonstrating the diagnostic benefits of CRD in birch pollen-related OAS compared to those of ImmunoCAP methods using crude allergens. The study demonstrated that the Bet v 1 homologous allergen Gly m 4 could also serve as a potential trigger of generalized severe reaction in birch pollen-allergic patients following soy consumption. CAP analysis of the sera of 20 patients from a birch pollen environment with reported soy allergy showed that most samples (15/20) were negative for soy-specific IgE, whereas 19/20 sera were found to contain specific IgE to Gly m 4, suggesting that Gly m 4 ImmunoCAP had greater sensitivity than ImmunoCAP using crude soy allergens [11]. These observations together with our current findings indicate that CRD analysis is more sensitive than the conventional ImmunoCAP method using crude allergens and therefore might constitute a superior method for the diagnosis of birch pollen-related OAS. However, the diagnostic

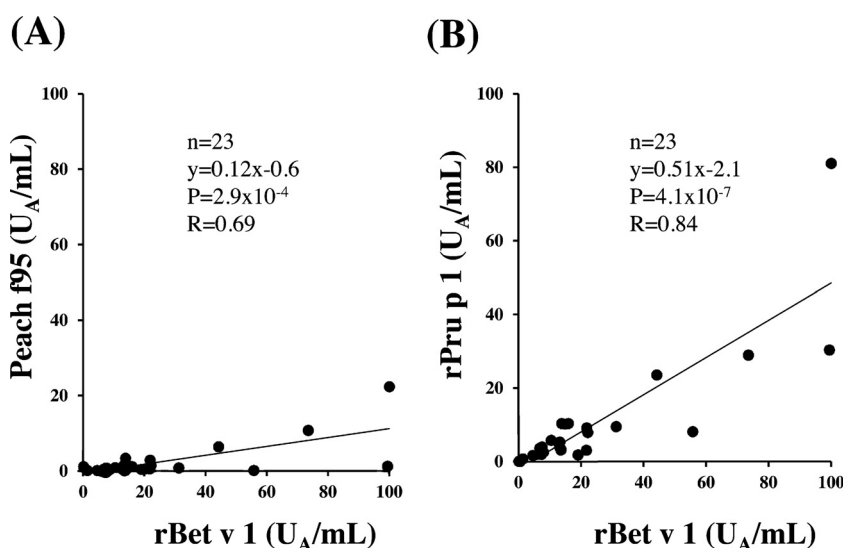


Fig. 5. Linear regression analysis of IgE values against rBet v 1 IgE values and peach specific IgE values. (A) Unfractionated peach extract (f95) versus rBet v 1; the slope of the regression line, 0.12. (B) Recombinant PR-10 allergen (rPru p 1) versus rBet v 1; the slope of the regression line, 0.51.

specificity of CRD analysis may be low, because CRD analysis on sera from our previous study [4] revealed that 13 of 20 sera from pollen-allergic patients without OAS had specific IgE against rMal d 1 and rPru p 1, suggesting the diagnostic specificity was only 35%. The diagnosis of OAS includes skin prick testing and oral food challenge tests in addition to serological diagnosis; however, these tests pose the risk of systemic side effects such as anaphylaxis. Although CRD is not a perfect method for diagnosing food allergies, clinicians are becoming increasingly aware of the potential uses of CRD [3].

In summary, component-resolved IgE antibody assays have great potential for improving the detection of a patient's sensitization profile in birch pollen-related OAS against Rosacea fruits. The use of CRD also improves the understanding of clinically relevant cross-reactive allergen components from aeroallergen sources and foods.

Conflict of interest

No one has any conflicts of interest to declare.

Acknowledgments

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