

Different IgE Reactivity Profiles in Birch Pollen-Sensitive Patients from Six European Populations Revealed by Recombinant Allergens: An Imprint of Local Sensitization

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Key Words

Bet v 1 · Bet v 2 · Bet v 4 · Birch pollen · Immunoblotting · Pharmacia CAP System · Recombinant allergens

Abstract

Background: Sensitivity to birch pollen allergens is a common feature among European patients with seasonal pollen allergy. In this in vitro study, we examined the specific serum IgE binding profiles to individual birch pollen allergens in birch-sensitive patients from six European populations. **Methods:** The study included 242 patients from Finland, Sweden, Austria, France, Switzerland and Italy. All suffered from seasonal rhinoconjunctivitis and/or asthma. Their sera were analyzed for specific IgE reactivity to individual birch pollen allergens (recombinant Bet v 1, Bet v 2 and Bet v 4) and natural birch pollen extract using Pharmacia CAP System™ and immunoblotting. **Results:** Almost all Finnish, Swedish and Austrian sera contained IgE specific for Bet v 1 ($\geq 98\%$). Bet v 1-specific IgE antibodies were found in 90% of the French sera, and in 65 and 62% of the sera from Switzerland and Italy, respectively. Few Finnish (2%) and Swed-

ish (12%) patients had IgE to Bet v 2, while Bet v 2 reactivity was more common in the other populations (20–43%). Reactivity to Bet v 4 was rare in all populations (5–11%) except for the Italian patients, in whom 3 of 11 sera were positive (27%). The immunoblot results supported the specific IgE profiles obtained with Pharmacia CAP System showing a broader IgE reactivity profile in patients from central and southern Europe as compared to northern Europe. **Conclusion:** Component-resolved allergy diagnosis with recombinant allergens reveals that the IgE reactivity profiles to individual birch pollen allergens vary between European populations. This observation may be explained by sensitization to different allergen sources and will have an impact on allergen-specific prevention and therapy strategies.

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Introduction

Allergy to pollen of white birch (*Betula verrucosa*) is beside grass pollen allergy one of the most common type I allergies in Europe [1]. Studies have shown that the preva-

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lence of IgE antibodies to birch pollen extract is between 5 and 54% in pollinosis patients in western Europe [1]. The IgE reactivity profile to individual birch pollen allergens has been studied by immunoblotting [2]. However, immunoblotting is only semiquantitative and individual allergens might be difficult to identify. In vitro assays based on single allergens for quantitative IgE measurements have so far been difficult to obtain due to the lack of defined reagents.

The molecular and biochemical characterization of allergenic proteins has made considerable progress since the application of cDNA cloning techniques. Complete nucleotide sequences and deduced amino acid sequences of cDNA clones coding for several birch pollen allergens have been reported [3–7]. The cDNAs have been expressed in bacteria as recombinant allergens with retained IgE-binding capacity [8, 9]. Consequently, they can be used in in vitro assays for IgE analysis [10, 11].

Bet v 1 is a major birch pollen allergen of 17 kD that is recognized by serum IgE of more than 96% of Austrian birch pollen-allergic patients [2]. Bet v 2 is a protein of 13–14.4 kD belonging to the family of profilins [12]. Profilins are homologous proteins found in all eukaryotic cells [4, 12]. About 10–20% of birch pollen-allergic patients have been shown to have IgE antibodies recognizing Bet v 2 [9, 13]. The 24-kD allergen Bet v 3 represents a class of calcium-binding proteins and is recognized by IgE from about 10% of pollen-allergic patients [5, 14]. The calcium-binding protein Bet v 4 is a small allergen of only 9.3 kD that has been shown to react with IgE in approximately 20% of pollen-allergic individuals [6]. The recently cloned Bet v 6 is a phenylcoumaran benzylic ether and isoflavonoid reductase of 35 kD [7]. It has been reported to bind IgE in sera from 12% of birch pollen-allergic patients. Furthermore, a cyclophilin from birch pollen has been purified and identified as an allergen [15]. It was named Bet v 7 and was recognized by serum IgE from up to 21% of birch pollen-allergic patients. Another novel birch pollen allergen (Bet v 8) of 65 kD has been cloned that shares sequence homology with plant pectin esterase [16].

The IgE reactivity to individual grass pollen allergens has been shown to differ between populations of grass pollen-allergic patients living in different countries [17]. Our preliminary data, recapitulated from a presentation at the 21st Symposium of the Collegium Internationale Allergologicum, indicated that this could also be true for patients sensitive to birch pollen [18]. In this study, we present the final analysis of IgE reactivity profiles to individual birch pollen allergens for pollinosis patients living

in six different areas of western Europe. Using the Pharmacia CAP System™, sera from 242 birch-sensitive patients were analyzed for IgE with specificity for three selected recombinant allergens: Bet v 1, Bet v 2 and Bet v 4. The selection of recombinant allergens was based on their reported IgE reactivity among birch-pollen-allergic patients and their availability when the study was performed. Some representative sera were also studied by IgE immunoblotting.

Material and Methods

Patients

Two hundred and forty-two sera were collected from six different centers including patients with a case history of seasonal pollen allergy including symptoms of rhinoconjunctivitis and/or asthma. The patients were living in or near the cities of Helsinki (Finland), Skövde (Sweden), Vienna (Austria), Strasbourg (France), Lausanne (Switzerland) and Milan (Italy). Criteria for the final selection to the present study were a positive skin prick test to birch pollen and positive IgE reactivity to birch pollen extract (>0.35 kU_A/l) as measured by the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). Most of the patients were also sensitive to additional pollen and had symptoms associated with fruits and/or nuts. The patients, except the subjects from Austria, have been described in detail in previous reports [13, 19–22]. A summary of the patient data is presented in table 1.

Recombinant Allergens

Purified recombinant Bet v 1 and Bet v 2 were purchased from Biomay (Vienna, Austria). Purified recombinant Bet v 4 was kindly provided by Dr. Jonas Lidholm (Pharmacia Diagnostics). Bet v 4 was expressed as a nonfusion protein in *Escherichia coli*, and purified essentially as previously described [23], but with the use of immobilized metal affinity chromatography instead of chromatofocusing.

Measurement of Specific IgE to Birch Pollen Extract, Recombinant Bet v 1, Bet v 2 and Bet v 4

Serum levels of allergen-specific IgE were measured by the Pharmacia CAP System according to the instructions of the manufacturer. ImmunoCAP™ with birch pollen extract, recombinant Bet v 1 and Bet v 2 are commercially available. Experimental ImmunoCAP carrying recombinant Bet v 4 was prepared according to the standard methods of Pharmacia Diagnostics, and a corresponding test is now commercially available. The immunoreactivity of the recombinant allergens was confirmed by inhibition studies during validation of the Bet v 1, Bet v 2 and Bet v 4 ImmunoCAP (data not shown). All 242 sera were analyzed in the same laboratory in Uppsala. Due to limited serum volumes, only 210 sera were assessed for Bet v 4 reactivity. Positive and negative controls were included in the analyses. The cutoff value of the assay was 0.35 kU_A/l.

Immunoblotting Experiments

Freeze-dried extract of birch pollen (*B. verrucosa*, Allergon, Ängelholm, Sweden) was dissolved in water and treated with sodium dodecyl sulfate (SDS) and mercaptoethanol at 100°C. The extract was loaded on a continuous 7.5–20% gradient gel with a 6% stacking

Table 1. Patient characteristics including data from the original studies

Origin of population	Patients in original study	Age years	Sex (male/female)	Birch pollen-sensitive ¹	Ref. No.
Finland	46	20 (12–49)	25/21	41	19
Sweden	47	34 (17–58)	23/24	42	20
Austria	50	62 (6–85)	30/20	50	NP
France	51	34 (13–58)	29/22	51	13
Switzerland	145	33 (14–65)	83/62	37	21
Italy	71	33 (10–63)	40/31	21	22

Age is given as mean with the range in parentheses. NP = Not published.

¹ Criterion for inclusion in the present study.

zone (about 600 µg protein/cm) for separation in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using PROTEAN II xi vertical Slab Cell (Bio-Rad, Hercules, Calif., USA) [24]. Directly after SDS-PAGE the proteins were transferred to a nitrocellulose membrane by electroblotting using the Trans-Blot electroblotting system (Bio-Rad) [25]. Remaining free binding sites were blocked with phosphate-buffered saline containing 0.3% dextran and 0.1% Tween 20. The same buffer was also used for dilution of serum samples. The nitrocellulose membrane was then cut into 5-mm strips. Each strip was incubated overnight at 4°C with 2 ml of patient sera diluted to 2–3 kU_A/l of birch pollen-specific IgE (calculated from in vitro measurements). Pools of sera from birch pollen-allergic patients were included as positive controls. Sera lacking IgE to birch pollen extract were included as negative controls. The strips were rinsed with washing solution (0.9% NaCl, 0.5% Tween 20) and incubated for 4 h at room temperature with ¹²⁵I-labeled anti-human IgE antibodies (Pharmacia CAP System Specific IgE RIA tracer; Pharmacia Diagnostics). The strips were then washed as above, dried and subjected to autoradiography for 70 h at –70°C using high-performance autoradiography film (Hyperfilm MP, Amersham Pharmacia Biotech, Uppsala, Sweden) in an intensifying screen cassette. The developed films were scanned and evaluated with Pharmacia ImageMaster System (Amersham Pharmacia Biotech). The detection limit for IgE to an individual allergenic component was less than 0.1 kU_A/l.

Inhibition experiments were performed to identify the position of Bet v 2 and Bet v 4 in the immunoblot. Selected patient sera were diluted 1:3 to 1:10 and preadsorbed for at least 6 h at +4°C with 5–50 µg/ml recombinant Bet v 2 or 10 µg/ml nitrocellulose-blotted recombinant Bet v 4 prior to incubation with nitrocellulose strips coated with SDS-PAGE-separated birch pollen extract. To confirm the specificity of the inhibition, sera were also incubated with buffer or irrelevant proteins. Similar incubation times and washing procedures as above were used for the inhibition experiments. The immunoblotting experiments were performed in Uppsala except for the Bet v 4 inhibition experiment which was performed in Vienna.

Statistical Analysis

Correlation between IgE concentrations was evaluated by Spearman's rank correlation test. χ^2 test was used to identify significant differences in prevalence between the populations. The statistical analyses were performed using the software GraphPad Prism version 2.0 (GraphPad Software, San Diego, Calif., USA).

Table 2. Percentage of pollinosis patients with IgE reactivity to the recombinant allergens Bet v 1, Bet v 2 and Bet v 4 as assessed by Pharmacia CAP System

Population	Bet v 1, %	Bet v 2, %	Bet v 4, %
Finland	100	2	5
Sweden	98	12	8
Austria	98	30	11
France	90	20	6
Switzerland	65	43	7
Italy	62	33	27
p value	<0.0001*	0.0002*	0.27

The numbers of patients in each population analyzed for IgE reactivity (>0.35 kU_A/l) to recombinant Bet v 1 and Bet v 2 were: 41 (Finland), 42 (Sweden), 50 (Austria), 51 (France), 37 (Switzerland) and 21 (Italy). Due to limited serum volumes, fewer patients were analyzed for IgE reactivity to recombinant Bet v 4. The corresponding numbers were: 39 (Finland), 37 (Sweden), 45 (Austria), 50 (France), 28 (Switzerland) and 11 (Italy). Significant differences (*p < 0.05) in prevalence between the populations as evaluated by χ^2 test.

Results

Specific IgE to Recombinant Bet v 1, Bet v 2 and Bet v 4

The serum levels of specific IgE to birch pollen extract varied between the six study populations, and so did the IgE reactivity profiles to the recombinant birch pollen allergens Bet v 1, Bet v 2 and Bet v 4 (fig. 1). All Finnish birch pollen-sensitive patients had IgE antibodies against the major birch pollen allergen Bet v 1 (fig. 1, table 2). The frequency of individuals with Bet v 1-specific IgE antibodies among the birch pollen-sensitive patients in Sweden, Austria and France was 90–98% (table 2), while the

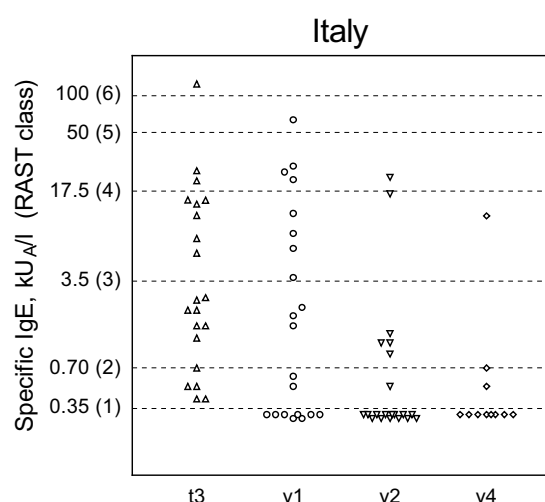
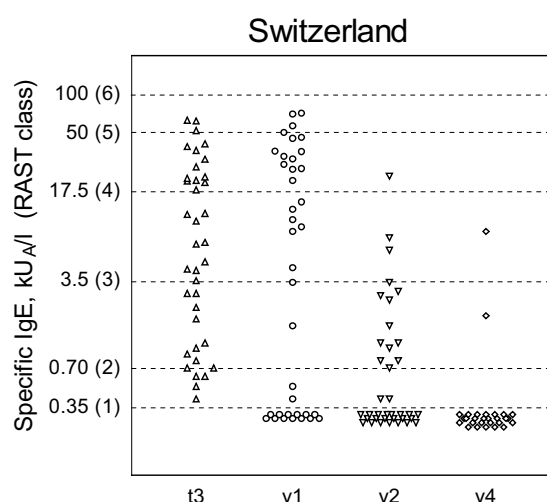
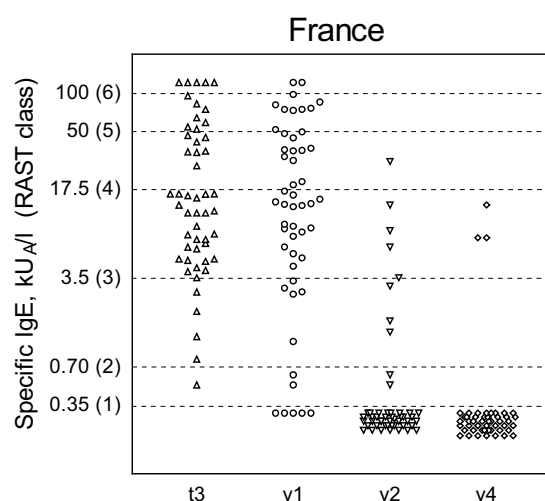
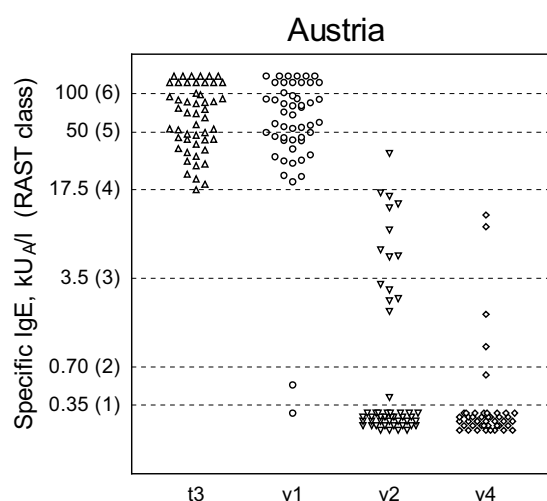
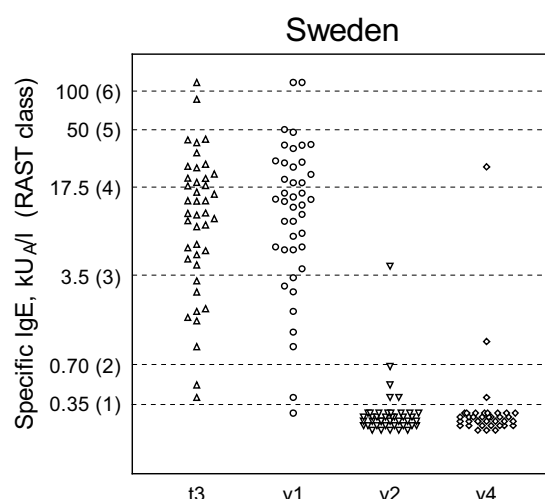
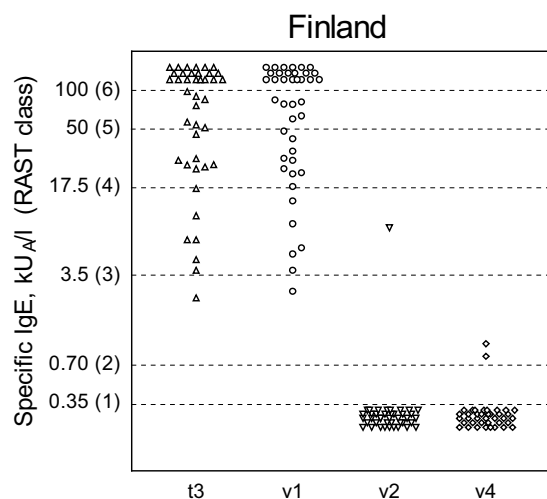


Table 3. Percentage of pollinosis patients with IgE reactivity to different combinations of recombinant Bet v 1, Bet v 2 and Bet v 4 as assessed by Pharmacia CAP System

Population	n	Bet v 1: +	+	+	+	+	–	–	–	–
		Bet v 2: –	+	–	+	+	+	+	–	–
		Bet v 4: –	–	+	+	+	–	+	+	–
Finland	39	92	3	5	0	0	0	0	0	0
Sweden	37	86	5	3	5	0	0	0	0	0
Austria	45	67	20	4	7	2	0	0	0	0
France	50	80	10	0	2	4	2	2	0	0
Switzerland	28	46	29	0	4	11	0	4	7	7
Italy	11	46	0	18	9	27	0	0	0	0
Total	210	74	12	3.3	3.8	4.3	0.48	0.95	0.95	0.95

Positive (+) and negative (–) IgE reactivity is indicated (cutoff value, 0.35 kU_A/l). Figures represent percentage.

percentage was considerably smaller in the study populations of Italy and Switzerland (62–65%). IgE reactivity to Bet v 2, on the other hand, was rare in Finland and Sweden (2 and 12%, respectively), but was more abundant in the other populations. Sensitivity to Bet v 4 was generally uncommon, but Bet v 4-specific IgE antibodies were found in 3 of the 11 Italian sera analyzed (27%).

Fifteen of 54 (28%) Bet v 2-sensitive patients and 3 of 18 (17%) Bet v 4-sensitive patients did not have IgE antibodies to Bet v 1. The majority of these Bet v 1-nonsensitive patients were from France (4 patients), Switzerland (7 patients) and Italy (4 patients), and only 1 patient was from Austria (Bet v 2-sensitive). In the few Bet v 2- and/or Bet v 4-sensitive patients from Finland and Sweden, poly-reactivity to Bet v 1 was always present. Very few patients (3.8%) had IgE reactivity to all three recombinant birch pollen allergens (table 3).

Of all 242 birch pollen-sensitive patients, 214 (88%) were found to have IgE antibodies to recombinant Bet v 1 and 54 (22%) to recombinant Bet v 2. Two hundred and twenty-nine (95%) were found to have IgE antibodies to either recombinant Bet v 1 and/or Bet v 2. When the panel of recombinant birch pollen allergens was comple-

mented with Bet v 4, 208 of 210 (99%) sera analyzed were positive for IgE to at least one of the three recombinant allergens. The two negative sera, both from patients from the Swiss population, had low levels of IgE (0.6 and 2.1 kU_A/l) to birch pollen extract.

A strong correlation was found between the serum levels of IgE specific for birch pollen extract and for Bet v 1 ($r = 0.98$, $p < 0.0001$). A similar correlation was observed for each population when they were evaluated individually (data not shown). A weak, but significant, correlation was observed between the levels of IgE to birch pollen extract and IgE to Bet v 2 ($r = 0.40$, $p < 0.005$). No statistical correlation was found between IgE to birch pollen extract and IgE to Bet v 4 ($r = -0.15$, $p = 0.56$).

Immunoblotting

Representative patient sera from the Swedish ($n = 42$), Swiss ($n = 33$) and Italian ($n = 13$) populations were selected for further characterization by immunoblotting. Their IgE-binding profiles to distinct allergens present in birch pollen extract were evaluated with the help of Pharmacia ImageMaster System and presented as allergograms (fig. 2a–c). A summary of the frequencies of IgE reactivity to Bet v 1, Bet v 2 and Bet v 4 as assessed by immunoblotting is shown in table 4. All but three Swedish sera (93%) contained IgE specific for a 17-kD protein, which corresponds to Bet v 1. Only few of the Swedish sera contained IgE to proteins of lower molecular weight (≤ 12 – 15 kD), including Bet v 2 with a reported molecular weight of about 13–14.4 kD (fig. 2a, table 4). The approximate position of Bet v 2 was confirmed by inhibition experiments with recombinant Bet v 2. In some patient sera two bands

Fig. 1. The specific IgE levels to natural birch pollen extract (t3), recombinant Bet v 1 (v1), Bet v 2 (v2) and Bet v 4 (v4) in six European populations of birch pollen-sensitive pollinosis patients analyzed by the Pharmacia CAP System. The specific IgE levels corresponding to the lower limits of the RAST classes are indicated on the logarithmic y-scale. The numbers of sera analyzed for IgE antibodies against the recombinant allergens are given in table 2.

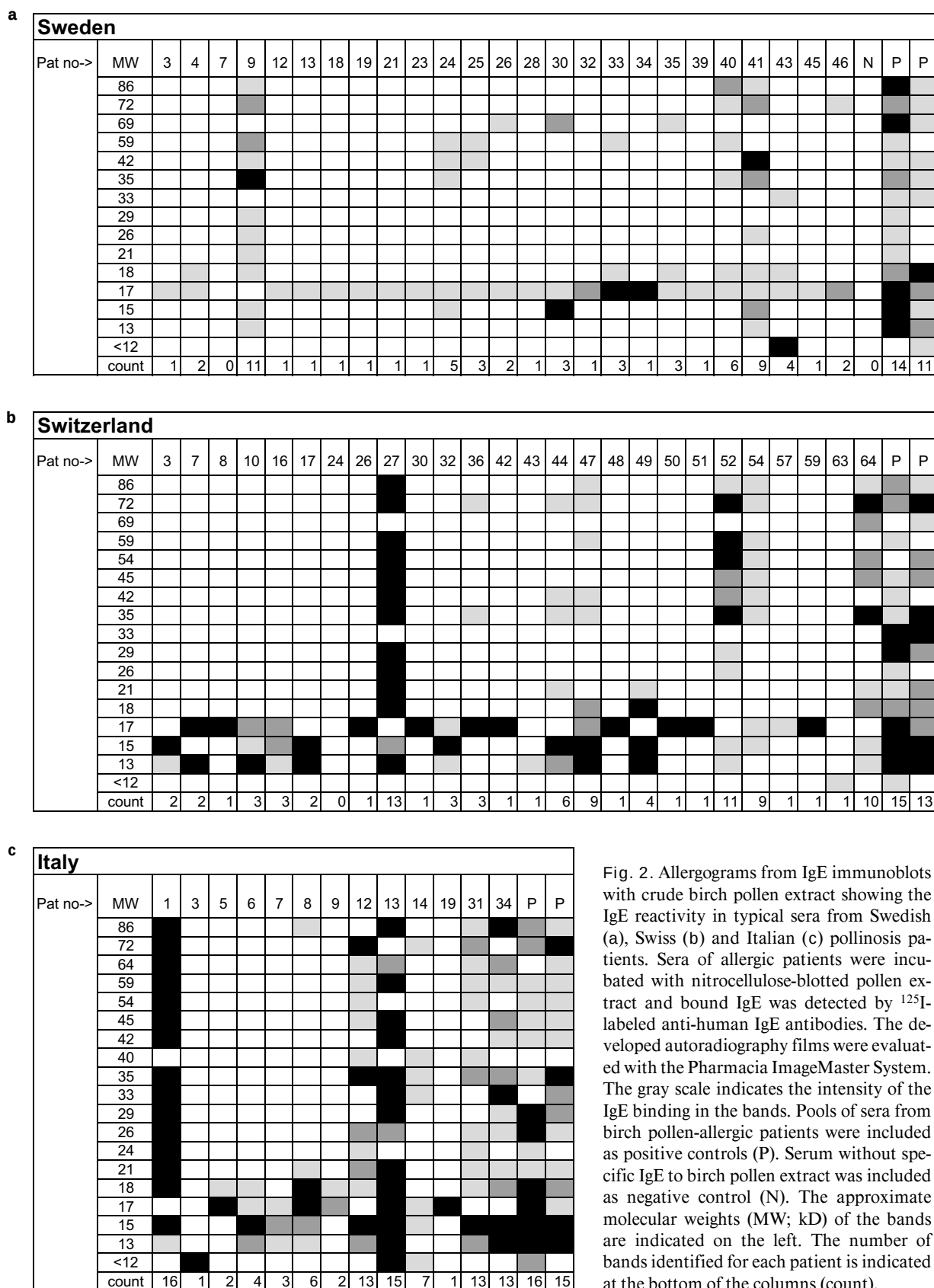


Fig. 2. Allergograms from IgE immunoblots with crude birch pollen extract showing the IgE reactivity in typical sera from Swedish (a), Swiss (b) and Italian (c) pollinosis patients. Sera of allergic patients were incubated with nitrocellulose-blotted pollen extract and bound IgE was detected by ^{125}I -labeled anti-human IgE antibodies. The developed autoradiography films were evaluated with the Pharmacia ImageMaster System. The gray scale indicates the intensity of the IgE binding in the bands. Pools of sera from birch pollen-allergic patients were included as positive controls (P). Serum without specific IgE to birch pollen extract was included as negative control (N). The approximate molecular weights (MW; kD) of the bands are indicated on the left. The number of bands identified for each patient is indicated at the bottom of the columns (count).

Pharmacia CAP System™												
Immunoblotting	a			b			c					
		+	−	total		+	−	total		+	−	total
	+	77.3%	2.3%	79.6%	+	23.9%	13.6	37.5%	+	5.8%	0%	5.8%
	−	4.5%	15.9%	20.4%	−	4.5%	58.0%	62.5%	−	2.9%	91.3%	94.2%
	total	81.8%	18.2%	100%	total	28.4%	71.6%	100%	total	8.7%	91.3%	100%
Bet v 1 Bet v 2 Bet v 4												

Fig. 3. Comparison of results from the Pharmacia CAP System and immunoblotting in sera from the Swedish, Swiss and Italian populations. Values are expressed as percentage of total sera analyzed for IgE reactivity to Bet v 1 (a; n = 88), Bet v 2 (b; n = 88) and Bet v 4 (c; n = 69).

were found at about 13–14 and 14–15 kD. These bands were selectively reduced by preincubating the sera with recombinant Bet v 2 (data not shown). Some of the Swedish sera also contained specific IgE to additional unidentified proteins with higher molecular weights.

The sera from Switzerland and Italy generally showed a more complex IgE-binding pattern on the immunoblot. IgE binding to Bet v 1 at 17 kD was less common (Switzerland: 70%, Italy: 62%) and several other IgE reactivities were more frequent than in the Swedish population, especially in the lower molecular weight range (≤ 12 –15 kD) (fig. 2b, c, table 4). Patient 17 in the Swiss population was negative for all three recombinant allergens in the Pharmacia CAP System, but the immunoblot revealed IgE reactivity to the two Bet v 2 bands at 13–15 kD.

Serum samples from 2 Swedish (5%), 2 Swiss (6%) and 3 Italian (23%) patients contained specific IgE antibodies recognizing a low molecular weight protein of less than 12 kD, suggesting that it could be the minor allergen Bet v 4. The corresponding band on the immunoblot was completely inhibited when the sera were preincubated with recombinant Bet v 4 (data not shown). Additionally, IgE binding to the ≤ 12 -kD band was only seen with sera that were positive for IgE to recombinant Bet v 4 in the Pharmacia CAP System.

Comparison between Immunoblotting and Specific IgE Measurements

Generally, the agreement between the results from the immunoblot and Pharmacia CAP System was good

Table 4. Percentage of pollinosis patients with IgE reactivity to the birch pollen allergens Bet v 1, Bet v 2 and Bet v 4 as assessed by immunoblotting

Population	n	Bet v 1, %	Bet v 2, %	Bet v 4, %
Sweden	42	93	12	5
Switzerland	33	70	58	6
Italy	13	62	69	23
p value		0.01*	<0.0001*	0.090

Significant differences (*p < 0.05) in prevalence between the populations as evaluated by χ^2 test.

(fig. 3). The agreement regarding assessed IgE reactivity was 93.2% for Bet v 1, 81.9% for Bet v 2 and 97.1% for Bet v 4.

Discussion

We show that the IgE reactivity patterns to individual allergens differ between birch pollen-sensitive patients living in different areas of Europe. While IgE antibodies to Bet v 1, as assessed by Pharmacia CAP System, were almost certainly present in patients from Sweden, Finland and Austria ($\geq 98\%$) and common in France (90%), they were present in less than 70% of the patients from Switzerland and northern Italy. Among the Bet v 1-sensitive

patients in all populations, there was a correlation between the serum levels of IgE to natural birch pollen extract and the IgE levels to recombinant Bet v 1. This confirms previous studies that Bet v 1 is a highly immunogenic protein and that recombinant Bet v 1 possesses a high IgE-binding capacity [26]. Regarding sensitivity to Bet v 2, the situation was different. IgE reactivity to Bet v 2 was rare in the Scandinavian patients, while it was more abundant in the patients living in central or southern Europe. There were, however, no major differences between the populations regarding sensitivity to Bet v 4, even if a trend towards a higher prevalence among the Italian patients was noted.

Analysis of sera from Sweden, Switzerland and Italy by immunoblotting confirmed that sensitivity to Bet v 1 was more predominant in the Swedish patients compared to the other two populations. In both the Swiss and Italian patients, however, it was rather common with IgE against Bet v 2 at 13–14.4 kD. Bands at 12 kD or less were also observed, but these bands were rare in all three populations studied. The ≤ 12 -kD band corresponded to IgE reactivity to Bet v 4, since this band could be completely inhibited by preincubating the sera with purified recombinant Bet v 4.

Our finding that the IgE reactivity profile to pollen allergens can differ between European populations is supported by another study including grass pollen-allergic patients [17]. We found no association between the magnitude of the IgE response to birch pollen extract and the IgE reactivity profile to the individual birch pollen allergens. The Scandinavian region was represented by two populations from Finland and Sweden. Even if the birch pollen-specific IgE levels in the two study groups were quite different, the IgE reactivity profiles of the Finnish and Swedish patients were similar.

The exposure to various pollens from trees, grasses or weeds varies greatly in different European regions due to their individual climate and flora [1]. To understand the differences in the IgE reactivity pattern to pollen allergens between populations, their normal exposure to various pollens can give an indication. Furthermore, the structural and immunological relationship between allergens of diverse allergen sources should be considered.

White birch belongs to the family Betulaceae. The pollen grains contain highly allergenic proteins and are the main cause of seasonal pollen allergies in northern and central Europe [1]. Trees belonging to the Betulaceae and Corylaceae families (order Fagales) have in recent years become popular ornamental plants in several countries, in which their natural distribution is rather limited [1, 27]. It

cannot be ruled out that planted birch or related trees in these areas act as initiators of new sensitization, which may explain the recent significant increase in sensitivity to Betulaceae pollen in northern Italy [27]. Variations in the expression of Bet v 1 in pollen from birch trees growing at different locations have been described [28], and the allergenic potency of separate batches of pollen extracts can differ [29]. Hence, it might be possible that a variation in the exposure of individual birch pollen allergens may have played a role for the IgE reactivity profiles observed in the present study.

We believe that the difference in the IgE reactivity pattern to birch pollen allergens between the populations in the present study could imply differences in the primary sensitization pathways. Cross-reactive IgE antibodies to birch pollen allergens and allergens from fruits, such as apple and peach [30–33], vegetables, such as carrot and celery [33–35], and nuts [36] are common among birch pollen-allergic patients. We cannot exclude that some patients in our study, who also suffer from allergy to fruits and nuts, may have developed cross-reacting IgE antibodies to birch pollen allergens due to primary sensitization to the food allergens. However, this is not very likely. Inhibition experiments have suggested that the primary sensitization occurs to birch pollen rather than to the homologous proteins in fruits or vegetables [34]. Important roles for both Bet v 1 and Bet v 2 in the induction of cross-reacting IgE antibodies in pollen-food allergy have been proposed [34]. Proteins homologous to Bet v 1 can be found in pollen from trees belonging to the order Fagales, but not in grass pollen, while the profilins, to which Bet v 2 belongs, are a ubiquitous panallergen [12]. Clinical symptoms when eating plant-derived food have been shown to be associated with sensitization primarily to Bet v 1 rather than to Bet v 2 [10, 13]. Other studies concerning Spanish patients have shown an association between profilin and allergy to fruits [31, 37]. The discrepancy between the studies could be explained by a lower exposure to Bet v 1 homologous allergens for people living in Spain compared to areas where trees of the order Fagales are common. Similarly, while patients from Sweden, Finland and Austria most probably have been exposed to high concentrations of birch pollen in their inhaled air during childhood and youth, other pollens more often cause primary sensitization in patients from Switzerland and Italy. Thus, it can be assumed that some pollinosis patients have cross-reactive IgE antibodies to birch pollen allergens as a result of an immune response primarily induced by homologous tree, grass or weed pollen allergens.

The major birch pollen allergen Bet v 1 is present in high amounts in birch pollen [38]. High birch pollen loads might be the reason why almost all birch pollen-sensitive patients in Sweden, Finland and Austria have IgE antibodies specific for Bet v 1. In northern Italy, olive trees (belonging to the family Oleaceae) are common and might contribute to a great deal of the air-borne pollens during spring [1, 27]. Some cross-reactivity could be expected between allergens from the two families of Oleaceae and Betulaceae. No Bet v 1-related protein has been found in olive tree pollen. However, Ole e 3 is a major olive pollen allergen and has been shown to be cross-reactive with Bet v 4 on the level of IgE specificity [6, 39]. Additionally, olive pollen profilin has been isolated that was able to inhibit IgE binding to natural Bet v 2 [40]. Due to the large structural homology within the profilin family, induction of cross-reacting IgE antibodies to profilins from most plants is common in pollinosis patients [12]. Sensitivity to Bet v 2 among birch pollen-allergic patients has been shown to be associated with grass pollen allergy [10, 13]. Thus, the lower frequency of Bet v 1 reactivity and higher frequency of Bet v 2 reactivity in the Swiss and Italian populations might indicate that these patients primarily have been sensitized to pollen allergens from other sources than from trees of the order of Fagales, for example from olive tree or grass. The French population from the Strasbourg area has probably been exposed to pollens from various plant species, and therefore these patients show a more mixed IgE reactivity pattern when analyzed in vitro with recombinant Bet v 1, Bet v 2 and Bet v 4.

Today, IgE sensitivity is investigated using in vitro assays or by studying early histamine-mediated skin reactivity using crude allergen extracts from natural sources. These natural sources might differ in composition between batches, and some allergens may be present only in low amounts, which can hamper an accurate allergy diagnosis [29, 41]. It has been suggested that recombinant allergens could replace the natural pollen extracts in the diagnosis of allergy [41]. Our study, including seasonal pollen-allergic patients from six European populations, indicates that only three recombinant allergens (Bet v 1, Bet v 2 and Bet v 4), in combination or as individual allergens, could be sufficient to identify 99% of birch pollen-sensitive patients. This is in accordance with previous observations [9]. Only 2 Swiss patients were negative for all tree recombinant allergens. Serum from one of them (patient 17) was analyzed by immunoblotting and was found to have IgE reactivity to the two Bet v 2 bands. However, the agreement between the test results obtained by Pharmacia CAP System and immunoblotting was gen-

erally good (fig. 3). The serum levels of IgE against Bet v 2 were in general lower than those against Bet v 1 (fig. 1). The less agreement between the two methods regarding assessed IgE reactivity to Bet v 2 could be attributed to a higher sensitivity of the immunoblotting technique. It was estimated to be less than 0.1 kU_A/l for individual allergenic components, while the used cutoff for Pharmacia CAP System was 0.35 kU_A/l.

Our aim was to study the presence of circulating allergen-specific IgE antibodies. Others have studied the allergenicity of recombinant birch pollen allergens and have shown that the recombinant allergens behave similarly in vivo and in histamine release assays as their natural counterparts [8, 13, 26]. A good correlation between serological assays and skin prick testing with recombinant birch pollen allergens has also been shown [13, 26].

In summary, our results demonstrate that birch pollen-sensitive allergic patients from various European areas differ in their IgE reactivity profile to individual birch pollen allergens (Bet v 1, Bet v 2 and Bet v 4). The finding that IgE reactivity to birch pollen extract can be caused by isolated sensitization to Bet v 2 and/or Bet v 4 without reactivity to the major birch pollen allergen Bet v 1 may be of clinical importance in specific immunotherapy. Since Bet v 2 and Bet v 4 are less well represented in therapeutic birch pollen extracts than Bet v 1, it is possible that patients without reactivity to Bet v 1 are less efficiently treated with birch pollen extract than those who are sensitized to Bet v 1. While the latter assumption cannot be proven at this moment, the dissimilarity in IgE reactivity profiles between populations most probably reflects differences in the primary sensitization due to variations in pollen exposure and is likely to have an impact on preventive and therapeutic considerations.

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