

IgE Reactivity Pattern to Timothy and Birch Pollen Allergens in Finnish and Russian Karelia

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Key Words

Birch pollen · Finland · IgE reactivity · Recombinant allergens · Russia · Timothy pollen · UniCAP[®]

Abstract

Background: Little is known about differences in IgE reactivity patterns to individual allergens in random populations. We studied the IgE reactivity profile to individual recombinant (r) and native (n) allergens in sera from subjects sensitized to timothy and/or birch pollen living in Finnish and Russian Karelia. **Methods:** Sera from IgE-sensitized adults were obtained from an epidemiological study on a random sample of 1,177 subjects. The IgE reactivity to pollen extracts and eight timothy (rPhl p 1, 2, 5, 6, 7, 11, 12 and nPhl p 4) and three birch pollen allergens (rBet v 1, 2 and 4) were analyzed with UniCAP[®]. **Results:** The levels of IgE antibodies to timothy and birch pollen were higher in Finnish (median 5.2, range 0.35 to >100 kU_A/l) than in Russian Karelia (median 1.8 kU_A/l, range 0.43–25.2 kU_A/l, $p < 0.01$). There was a significantly higher prevalence of IgE reactivity to three timothy pollen allergens in Finnish ($n = 57$) than in Russian Karelia ($n = 12$): rPhl p 2, 28 vs. 0%; rPhl p 5, 60 vs. 0%; rPhl p 6, 47 vs. 0%. The prevalence of IgE reactivity to the birch pollen allergens was similar in the two populations. IgE

reactivity to rPhl p 2, 5, 6 and 11 was associated with hay fever symptoms. The timothy-pollen-specific serum IgE levels and the numbers of IgE reactivities to individual allergens correlated significantly ($r_s = 0.87$, $p < 0.0001$).

Conclusions: The data indicate that timothy- and birch pollen-specific IgE levels are higher in Finnish compared to Russian Karelia. This is reflected in wider IgE reactivity to individual timothy pollen allergens in Finnish Karelia, including the major allergen Phl p 5, and increased pollen allergy.

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Introduction

IgE-mediated allergy is a growing problem, also in Finland [1]. The prevalence of atopy varies a lot between countries, and the increasing incidence of allergies are thought to be associated with urbanization and improved standard of living [2–4]. An epidemiological study including a random sample of 1,177 adult Finnish and Russian subjects living in the geographic area of Karelia has recently been published [5]. Finnish Karelia and Russian Karelia have similar climate and flora [6], but the inhabitants in Finnish Karelia have a much higher standard of living, better hygienic conditions and better health care than the Russian inhabitants. For example, according to the national official statistical information of Finland and Russia, the average floor area per person in 1998 was

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34.5 m² in Finland compared to 18.9 m² in the Russian Federation. The Russians also have a shorter life expectancy at birth (males, 60.8 years, and females, 73.1 years) compared to the Finnish people (males, 74.4 years, and females, 81.5 years). The population study showed that the prevalence of IgE sensitization and allergic diseases was significantly higher in Finnish than in Russian Karelia [5]. For example, the prevalence of serum IgE antibodies to timothy pollen was significantly higher in Finland than in Russia (8.6 vs. 3.9%), and the same pattern was seen for IgE antibodies to birch pollen (7.8 vs. 2.1%).

The IgE reactivity profiles to individual allergenic pollen components (allergens) vary between different populations of pollen-allergic individuals [7–9]. The reason may be differences in the primary sensitization pathways due to differences in pollen exposure between geographically and botanically different areas. It is well known that sensitization to pollen allergens belonging to certain protein families may induce cross-reactive IgE antibodies that bind to structurally related allergens in pollen from other plants. Our knowledge, however, is limited regarding IgE reactivity profiles to pollen allergens in randomly selected populations without pre-selection for seasonal pollen allergy.

The aim of this study was to assess the IgE reactivity profiles to individual recombinant (r) and native (n) allergens in sera from timothy- and birch-pollen-sensitized subjects from two randomly selected population samples living in Finnish and Russian Karelia. The association between self-reported hay fever symptoms and IgE reactivity to individual allergens was also studied.

Material and Methods

The study populations from which the sera were obtained have recently been presented elsewhere (the FINALLERGIA Study) [5]. Briefly, the sample aged 25–54 years was randomly drawn from the population registers of the North Karelia province, Finland (1,500 subjects) and the Pitkäranta district in the Republic of Karelia, Russia (750 subjects). Both populations were stratified on the basis of 10-year age groups and sex. The numbers of participants were 790 (53%) in Finnish Karelia, and 387 (52%) in Russian Karelia. Serum samples were collected from 772 subjects (51%) in Finnish and 385 (51%) in Russian Karelia. The participants filled out a questionnaire on previous or present hay fever symptoms during the pollen season. The study was approved by the ethic committee of the Finnish National Institute of Public Health.

The serum samples that have been analyzed in the present study were collected out of the pollen season (autumn) in 1998 from 95 Finnish and 17 Russian subjects who in the previous year had been found sensitized to timothy pollen and/or birch pollen by in vitro testing (Pharmacia CAP SystemTM, Pharmacia Diagnostics AB, Up-

psala, Sweden). The sera were stored at –20°C until analysis. IgE reactivity to timothy pollen extract (*Phleum pratense*) and the timothy pollen allergens rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5 (isoform 'b'), rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 12 were analyzed in 67 sera from Finnish Karelia and 15 sera from Russian Karelia using ImmunoCAPTM tests and UniCAP[®] 100 instrument (Pharmacia Diagnostics AB). IgE reactivity to birch pollen extract (*Betula verrucosa*) and the birch pollen allergens rBet v 1, rBet v 2 and rBet v 4 were analyzed in 60 sera from Finnish Karelia and 7 sera from Russian Karelia. Specific IgE concentrations above 100 kU_A/l (the upper limit of measurement of the assay) were set as >100 kU_A/l.

The Mann-Whitney U test (two-tailed p value) and the χ^2 test were used to recognize significant differences between Finnish and Russian Karelia, and between subjects with and without hay fever symptoms. Correlation between the timothy-pollen-specific serum IgE concentrations and the number of IgE antibody reactivities to individual timothy pollen allergens in all 82 sera analyzed was evaluated by Spearman's rank correlation test. Only p values less than 0.05 were considered significant.

Results

The serum levels of IgE antibodies to timothy and birch pollen were higher in Finnish Karelia (median 5.2 kU_A/l, range 0.35 to >100 kU_A/l) than in Russian Karelia (median 1.8 kU_A/l, range 0.43–25.2 kU_A/l, $p < 0.01$). In Finnish and Russian Karelia, the IgE levels to timothy pollen extract were 4.2 (0.35–94.3) and 1.9 (0.43–25.2) kU_A/l, respectively ($p < 0.05$). The corresponding figures for birch pollen extract were 6.1 (0.37 to >100) and 1.7 (0.49–18.4) kU_A/l ($p = 0.11$).

Twenty subjects (18%) that in 1997 had low specific IgE levels to timothy and/or birch pollen (median 0.42 kU_A/l, range 0.35–1.8 kU_A/l) were found to be negative when retested in 1998. Four of them were nevertheless positive for IgE antibodies to nPhl p 4 (median 0.72 kU_A/l, range 0.37–1.3 kU_A/l) and were therefore included in the prevalence estimation.

IgE antibody reactivity to timothy pollen allergens was more heterogeneous in Finnish Karelia than in Russian Karelia (table 1). In both populations, the most frequent IgE reactivity was to nPhl p 4 followed by rPhl p 1. IgE antibodies to the allergens rPhl p 2, rPhl p 5 and rPhl p 6 were significantly more common in sera from Finnish Karelia. The Russian subjects mostly (75%) had IgE antibodies to only one allergen (rPhl p 1 or nPhl p 4), whereas monosensitization was rather uncommon (23%) in Finnish subjects. Sensitization to one particular allergen was not more often associated with a sensitization to another particular allergen. The prevalence of IgE reactivity to individual birch pollen allergens was similar in the two populations (table 2). IgE reactivity to rBet v 1 dominat-

Table 1. Prevalence (%) and concentration (kU_A/l) of IgE antibodies to individual timothy pollen allergens

	n	ImmunoCAP test								
		TPE	rPhl p 1	rPhl p 2	nPhl p 4	rPhl p 5	rPhl p 6	rPhl p 7	rPhl p 11	rPhl p 12
Finnish Karelia	57	95 (4.2)	68 (2.8)	28 (4.4)	75 (1.8)	60 (3.6)	47 (1.5)	5.3 (2.4)	35 (1.1)	10 (0.87)
Russian Karelia	12	92 (1.9)	42 (2.3)	0 (NA)	68 (1.5)	0 (NA)	0 (NA)	0 (NA)	8.3 (0.57)	0 (NA)
p value		NS	NS	0.04	NS	0.0002	0.002	NS	NS	NS

Median specific IgE antibody levels are shown in parentheses. The study participants were selected from originally 67 Finnish and 15 Russian individuals with timothy pollen sensitization, identified in an epidemiological study. The criterion for selection was a positive result in at least one of the extract- or component-based tests shown in the table. TPE = Timothy pollen extract; NA = not available; NS = not significant ($p > 0.05$).

Table 2. Prevalence (%) and concentration (kU_A/l) of IgE antibodies to individual birch pollen allergens

	n	ImmunoCAP test			
		BPE	rBet v 1	rBet v 2	rBet v 4
Finnish Karelia	55	100 (6.1)	85 (17.9)	13 (0.53)	7 (1.2)
Russian Karelia	5	100 (1.7)	60 (10.5)	20 (3.9)	0 (NA)
p value		NS	NS	NS	NS

Median specific IgE antibody levels are shown in parentheses. The study participants were selected from originally 60 Finnish and 7 Russian individuals with birch pollen sensitization, identified in an epidemiological study. The criterion for selection was a positive result in at least one of the extract- or component-based tests shown in the table. BPE = Birch pollen extract; NA = not available; NS = not significant ($p > 0.05$).

ed the response, showing significantly higher specific IgE concentrations than rBet v 2 ($p < 0.001$) and rBet v 4 ($p < 0.05$).

Self-reported hay fever symptoms among the sensitized individuals were more common in Finnish Karelia (71%) than in Russian Karelia (31%, $p < 0.01$). Subjects with hay fever symptoms had significantly higher specific IgE levels to timothy pollen extract (median 5.8 kU_A/l, range 0.44–94.3 kU_A/l) than subjects without hay fever (median 1.6 kU_A/l, range 0.43–7.6 kU_A/l, $p < 0.01$). Hay fever symptoms were associated with IgE antibodies to especially rPhl p 5, but also to rPhl p 2, rPhl p 6 and rPhl p 11 (fig. 1). Subjects with hay fever did not have higher IgE antibody levels to birch pollen extract (median 7.3 kU_A/l, range 0.37 to >100 kU_A/l), than subjects without hay fever (median 3.1 kU_A/l, range 0.49 to >100 kU_A/l, $p = 0.34$), and the symptoms were not associated with sensitization to any particular birch pollen allergen (data not shown).

There was a strong correlation between the timothy-pollen-specific serum IgE levels and the number of IgE antibody reactivities to individual timothy pollen allergens in all 82 sera analyzed (fig. 2). Three positive sera in the extract-based test were negative for IgE antibodies to all eight timothy pollen allergens studied.

Discussion

The IgE reactivity to individual timothy and birch pollen allergens has been studied in different populations of pollen-allergic patients, and it varies between different geographic areas [7–9]. However, studies have not been performed on random populations, without pre-selection for symptomatic patients. In the present study, the IgE reactivity of individuals living in the Eastern Finnish province Karelia was compared with that in a corresponding group living on the other side of the Finnish-Russian border, the Republic of Karelia (Russian Karelia). Indi-

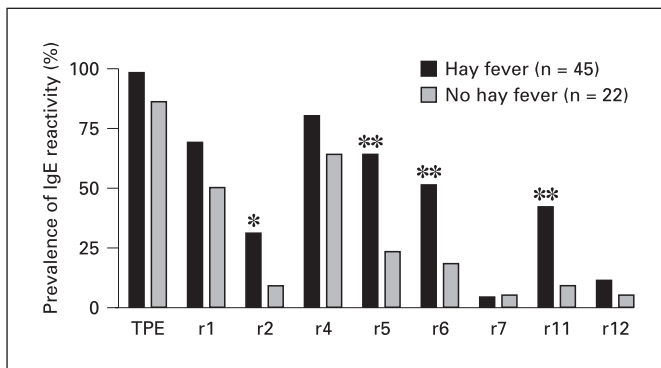


Fig. 1. IgE reactivity to timothy-pollen-extract (TPE)-based test and to recombinant rPhl p 1 (r1), rPhl p 2 (r2), nPhl p 4 (n4), rPhl p 5 (r5), rPhl p 6 (r6), rPhl p 7 (r7), rPhl p 11 (r11) and rPhl p 12 (r12) in sensitized individuals with and without self-reported hay fever symptoms. * $p < 0.05$, ** $p < 0.01$.

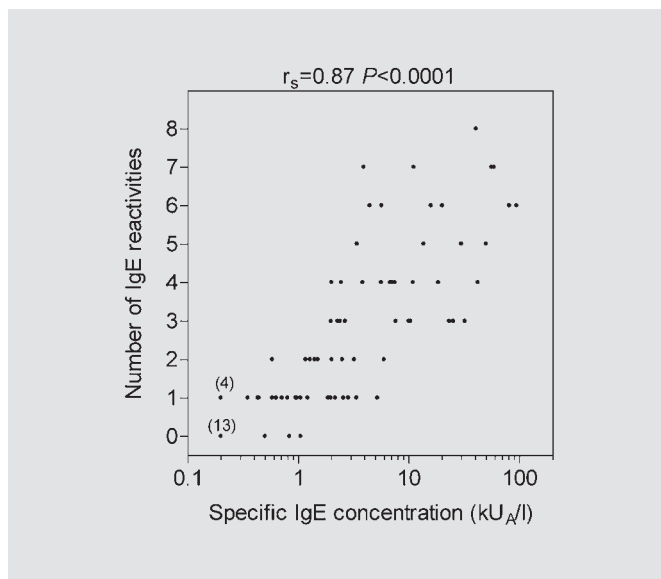


Fig. 2. Correlation between the serum levels of specific IgE antibodies to timothy pollen (extract-based test) and the number of IgE reactivities to individual timothy pollen allergens in the 82 sera analyzed. Cutoff value: 0.35 kU_A/l.

viduals with self-reported hay fever were compared with those without symptoms.

IgE sensitization to both timothy grass pollen and birch pollen, measured with conventional extract-based ImmunoCAP tests, was more frequently observed in Finnish Karelia than in Russian Karelia, as shown previ-

ously [5]. The present data also indicate that the serum concentrations of IgE antibodies to timothy and birch pollen are higher in randomly selected subjects from Finnish Karelia compared to Russian Karelia. The grass-pollen-sensitized individuals living in Finnish Karelia had more heterogeneous IgE reactivity profiles to the timothy pollen allergens studied than those in Russian Karelia. IgE reactivity to rPhl p 1 or nPhl p 4 was common in both Finnish Karelia and Russian Karelia, and was the most common IgE response in those who were sensitized to only one grass pollen allergen. As indicated in figure 2, only three sera positive in the timothy-pollen-extract-based test were negative for IgE reactivity to all eight individual timothy pollen allergens. These sera with rather low specific IgE levels might contain IgE antibodies to some unknown allergenic component not available for this study, or could have low levels of IgE antibodies below the cutoff value of the UniCAP assay to a few different timothy allergens.

In the present study, hay fever symptoms were found to be especially associated with IgE reactivity to rPhl p 5, but also to rPhl p 2, 6 and 11. IgE reactivity to rPhl p 2, rPhl p 5 and rPhl p 6 was not observed in the Russian group but was quite common in the Finnish population. For these three allergens, the difference between the populations was significant. Our finding that IgE binding to rPhl p 5 was absent in the Russian grass-pollen-sensitized individuals and associated with hay fever symptoms is striking. Phl p 5 is highly immunogenic and belongs to the group 5 grass pollen allergens. These allergens have been identified only in the Pooideae subfamily of grasses [10]. Phl p 5 is known as a major timothy pollen allergen, and the concentration of the Phl-p-5-specific IgE antibodies is often high [9, 11]. Phl p 6 has a 56–61% sequence homology to isoforms of Phl p 5 [12], and both allergens have common IgE binding epitopes [13]. It has been suggested that IgE binding to Phl p 6 in major part consists of cross-reacting IgE antibodies induced against Phl p 5 [10].

Phl p 4 was the allergen with the highest IgE-binding prevalence in this study. It is the only native allergen in the component-based tests, and carries 10–15% carbohydrates. It cannot be excluded that the high prevalence of Phl p 4 sensitization observed in this and other studies might be in part due to IgE antibodies binding so-called cross-reacting carbohydrate determinants [14]. Eight of 10 individuals with single IgE reactivity to nPhl p 4 had no self-reported hay fever symptoms. However, individuals with single IgE reactivity to rPhl p 1 were also over-represented in the group without symptoms compared to the hay fever group. The rPhl p 1 used in the present study

Table 3. Prevalence (%) of IgE antibody reactivity to timothy and birch pollen allergens in sensitized subjects from random populations (the present study) and in patients with seasonal pollen allergy (previously published data)

	Allergens										
	Phl p 1	Phl p 2	Phl p 4	Phl p 5	Phl p 6	Phl p 7	Phl p 11	Phl p 12	Bet v 1	Bet v 2	Bet v 4
Sensitized subjects ^a	64	23	74	49	39	4.3	30	8.7	83	13	6.7
Allergic patients ^b	~90	40–60	<80	65–85	60–70	~10	~65	15–30	>95	10–20	~20

^a Subjects from Finnish and Russian Karelia.

^b Data from other studies [reviewed in ref. 7 and 10].

is produced in *Escherichia coli* and is therefore lacking carbohydrates. Phl p 1 belongs to the group 1 grass pollen allergens that are found in pollen from most grass species and are considered to be the most important grass pollen allergen [10].

A strong correlation was observed between the concentrations of IgE antibodies to timothy pollen extract and the number of IgE reactivities to individual allergens. Similar correlations have recently been shown for both the IgE antibody level [10] and the magnitude of skin prick reactions to timothy pollen extract [15].

The IgE reactivity profiles to the birch pollen allergens Bet v 1, Bet v 2 (profilin) and Bet v 4 were similar between the two populations. IgE reactivity to Bet v 1 was observed in 83% of the Karelian subjects, which is lower than in previous reports on birch-pollen-allergic patients living in Northern and Central Europe (including Finland) in which a prevalence of more than 95% was reported [7, 16]. Similar differences between our study and others were observed for most allergens (table 3). Generally, the earlier data originate from studies on symptomatic patients living in the Western hemisphere. The dissimilarity regarding the prevalence of IgE reactivity to some of the timothy and birch pollen allergens between the present study and the other studies might be explained by the selection criteria. The Karelian subjects were now selected out of a random sample due to their IgE sensitization to timothy or birch pollen without selection of clinical symptoms. This might affect the prevalence results. It is believed that clinical symptoms to birch pollen are especially associated with Bet v 1 sensitization. This major birch pollen allergen belongs to a protein family expressed in trees of the order Fagales and is a marker of original birch pollen sensitization [17]. Profilins and Bet-v-4-homologous allergens in pollen from grasses, weeds and unrelated trees may induce IgE antibodies that cross-

react with Bet v 2 and Bet v 4. The clinical relevance of these IgE antibodies is not clear [17, 18]. In the present study no significant difference was observed concerning the prevalence of IgE reactivity to rBet v 1, rBet v 2 and rBet v 4 between individuals with reported hay fever symptoms and those without symptoms. However, no information was available on the time of the year when the subjects had their hay fever symptoms. Some of the subjects with self-reported hay fever, but without Bet v 1 sensitization, might well have an isolated grass pollen allergy.

The discrepancies observed between Finnish Karelia and Russian Karelia are not a consequence of differences in pollen exposure. The study populations live in two geographically adjacent communities close to the Finnish-Russian boarder with similar climate and flora [6]. The level of airborne pollutants should not much differ between the two communities [5]. The effect of differences in the genetic background of the two populations cannot be ruled out, although the development of pollen allergy seems to be more connected to allergen exposure and lifestyle than genetic background [19].

The prevalence of IgE sensitization and allergic diseases has increased in Western Europe during the last decades [20, 21] and the allergen-specific IgE antibody levels have increased [1]. Thus, the higher pollen-specific IgE levels in the Finnish population and the associated heterogeneous IgE reactivity pattern to individual pollen allergens may reflect the progress of IgE-mediated allergy in Western compared with Eastern Europe [5]. Interestingly, our data indicate that IgE reactivity to Phl p 5 might be a marker of allergic development and clinical symptoms in a population. This is supported by our unpublished data that the prevalence of sensitization to particularly Phl p 5 has increased since the 70s in Finland, together with an increase in allergy and asthma [22].

Finnish and Russian Karelia differ significantly in social, economic and lifestyle terms. However, the factors concerning lifestyle and standard of living on the IgE reactivity pattern are beyond the scope of the present study and should be investigated further.

In summary, our data, which should be regarded as preliminary because of a relatively low number of sensitized subjects especially in Russian Karelia, indicate that timothy- and birch-pollen-specific IgE levels are higher in Finnish compared to Russian Karelia. This is revealed in broader IgE reactivity to individual timothy pollen allergens in Finnish Karelia, including IgE reactivity to the

major allergen Phl p 5, and increased prevalence of pollen allergy. The dissimilarities between the two geographically adjacent areas may reflect the change in sensitization in Western Europe which is only starting to take place in Eastern Europe.

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