

Original article

Antibody profiles and self-reported symptoms to pollen-related food allergens in grass pollen-allergic patients from northern Europe

Background: Most studies on pollen-related food allergy have so far focused on the association of birch/weed pollen allergens and plant food allergy. The aim of this study was to elucidate the allergen spectrum among a group of grass pollen-allergic patients from northern Europe and to relate the results to clinical histories of pollen-related food allergy.

Methods: Fifty-eight grass pollen-allergic patients answered a questionnaire regarding allergy to foods. Blood samples were taken to test IgE-reactivity to a large panel of pollen allergens and pollen- and nonpollen-related food allergens using crude allergen extracts and recombinant and native allergens.

Results: Three different groups of grass pollen-allergic patients were identified according to their IgE antibody profile: a grass pollen group only (19%), a grass and tree pollen group (29%) and a grass, tree and compositae (pan-) pollen group (48%). No sensitization to Bet v 1 as well as almost no IgE to plant food was observed in the grass pollen group. In contrast, nearly all patients in the two tree-related groups had IgE to Bet v 1, which reflected the high frequency of adverse reactions to typical birch-related food in these groups. Only four patients belonging to the pan-pollen group displayed IgE to profilin Phl p 12/Bet v 2. Patients in the pan-pollen group reported significantly more symptoms to food allergens compared with patients in the two other groups. The most frequently reported symptom was the oral allergy syndrome.

Conclusions: Sensitization to grass pollen alone is rare among grass pollen-allergic patients from northern Europe. The majority of patients are in addition sensitized to birch (Bet v 1), which seems to be closely related to their pollen-derived food allergy. The study highlights the advantage of using well-defined allergen molecules for the diagnosis of cross-reactivity between pollen and food allergens.

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Grass pollen is a significant elicitor of IgE-mediated allergy worldwide (1). Approximately 40% of allergic individuals show IgE reactivity to this allergen (1, 2). The dominating source of grass pollen allergen in northern and central Europe is timothy grass (*Phleum pratense*) (3). Molecular and biochemical characterization of *P. pratense* has revealed several allergen components (e.g. Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p 12) of which Phl p 1, Phl p 2, Phl p 4 and Phl p 5 have been shown to be major allergens (4–6). Phl p 12 belongs to the profilin family and Phl p 7 is a calcium-binding allergen. Phl p 12 is one of the most frequently studied allergens, representing a cross-reactive plant pan-allergen. It is, by shape and function, related to profilins of other plants, e.g. weeds, trees and some food (7).

Patients suffering from pollen allergy often display symptoms [urticaria, angioedema, rhinoconjunctivitis,

asthma, anaphylaxis, and the oral allergy syndrome (OAS)] (8–13) after consuming fresh fruits or vegetables (12, 14, 15). This is due to the existence of highly cross-reactive allergens in a variety of pollens, fruit and vegetables (15). The majority of pollen-related reactions to fruit has been shown to be associated with birch pollen allergy (16, 17). However, birch pollen-related reactions to fruits/vegetables such as apple, peach, and peanuts have also been found in a small number of grass pollen-allergic patients (12, 16). Although mugwort-allergic patients without birch pollen allergy are few, the presence of IgE antibodies has been reported to birch pollen-related foods, such as celery, carrot and spices (16). The frequency of pollen-related fruit allergy also varies depending on geographic region and dietary habits (16, 18, 19).

Pollen and food share some cross-reactive structures (e.g. cross-reactive carbohydrate determinants [CCD]) and

some families of proteins (e.g. profilins, pathogenesis-related and lipid-transfer proteins), but their clinical relevance has been much debated (20–22). On the one hand, it is argued that related allergens can cause unexpected clinical reactions, and on the other that these structures may cause sensitization without clinical manifestation, which may be a concern in diagnosing the patients (23, 24).

By using well-defined native and recombinant allergens from birch and grass pollen it has been possible to characterize the complex reactivity profile of allergic patients. Some of these allergen components have been shown to have homologues in a variety of vegetables and fruits (25, 26). IgE-reactivity to highly cross-reactive allergens allows us to identify patients with broad sensitization and may also explain why some patients sometimes display symptoms when they come in contact with allergens that are not obviously related (24, 25, 27).

As most studies on pollen-related food allergy so far have focused on the association of birch/weed pollen allergens and plant food allergy, the objective of this study was to elucidate the allergen spectrum among a group of grass pollen-allergic patients from northern Europe and to relate the results to clinical histories of pollen-related food allergy. Fifty-eight grass pollen-allergic patients underwent a medical examination and answered a detailed questionnaire regarding pollen-related food allergy and blood samples were taken. *In vitro* analyses were performed against a large panel of pollen allergens and pollen- and nonpollen-related food allergens utilizing natural allergen extracts as well as recombinant and native pollen allergens.

Methods

Patients

Fifty-eight grass pollen-allergic patients [35 males, mean age 33 years (range 18–52) and 23 females, mean age 32 years (range 19–56)] were recruited from the Allergy Clinic at the Karolinska University Hospital and the Danderyd Hospital, Stockholm. They had all undergone a medical examination including blood sampling. The selection was based on a positive case history of grass pollen allergy and IgE antibodies to timothy grass pollen extract (UniCAP® System; Pharmacia Diagnostics, Uppsala, Sweden). Food-related symptoms were verified by patient's case history taken using a questionnaire. All patients had a diagnosis of rhinoconjunctivitis and in addition 19 (33%) had asthma to airborne allergens. None of the patients had previously undergone specific immunotherapy. Serum samples were obtained from each subject. All participating patients provided informed consent.

Questionnaire

The patients were given a questionnaire with 36 questions about age, gender, smoking habits, current medication and symptoms to airborne allergens, grass pollen, tree pollen (birch, alder and oak), Compositae pollen (mugwort, dandelion and marguerite), latex and various food allergens, thirteen related to and three unrelated to

pollen. The patients were requested to list time lapse between ingestion of food and occurrence of six different types of symptoms. They also described and specified the duration of symptoms related to grass pollen. Occurrence and duration of symptoms after intake of food allergens was also listed in the questionnaire (e.g. OAS, asthma, rhinoconjunctivitis, oedema, urticaria and eczema).

Determination of allergen-specific IgE

Determination of IgE antibodies to different allergens was carried out in all sera ($n = 58$) using UniCAP® System according to the manufacturer's recommendations. The allergens employed were crude allergen extracts such as tree pollen (birch, alder and oak), Compositae pollen (mugwort, dandelion and marguerite), a mix of animal dander, a mix of moulds, house dust mite, and 26 different pollen- and nonpollen-related food allergens (Table 1). The sera were also tested for reactivity to seven recombinant (r) timothy grass pollen allergens and one native (n) glycosylated timothy grass pollen component (rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11 and rPhl p 12) and recombinant birch pollen allergens (rBet v 1, rBet v 2 and rBet v 4) (Table 2). All samples were considered positive when the IgE antibody levels exceeded 0.35 kU_A/l as recommended by the manufacturer.

Pollen counts

To get an understanding of the pollen exposure over a long period of time (15 years) in Stockholm, airborne pollen counts (birch, grass and mugwort) were evaluated. The pollen counts had been monitored daily during 1988–2003 by the Palynological Laboratory, Swedish Museum of Natural History, Stockholm, Sweden, as previously described (28). The values of annual total are expressed on the basis of pollen concentrations (pollen/m³).

Statistical analysis

The exact median rank test was used to assess the significance of difference in the means between the groups. Fisher's exact test was used to evaluate differences in reported symptoms between the groups. A P -value < 0.05 was considered as statistically significant. In the statistical analysis SASTM Release 6.12 software (SAS Institute Inc., Cary, NC, USA) was used.

Ethical considerations

The Ethics Committee at the Karolinska Institutet, Stockholm, approved the study.

Results

Sensitization profiles to natural allergen extracts

The serological analyses identified three different groups of grass pollen-allergic patients (Table 1). The first group comprised patients sensitized to timothy grass pollen only ($n = 11$) and was unexpectedly small (19%). The second group comprised 17 patients (29%) sensitized to timothy grass and tree pollen (birch and/or alder and/or oak) and the third group comprised 28 patients (48%) sensitized to timothy grass, tree and Compositae pollen (mugwort

IgE antibody profile and symptoms in pollen-allergic patients

Table 1. Comparison of three groups of pollen-allergic patients and their sensitization to plant food and other allergens expressed as range and median of specific IgE-values (kU_A/l) for each allergen

Allergen	Grass (<i>n</i> = 11)			Grass-tree (<i>n</i> = 17)			Pan-pollen group (<i>n</i> = 28)		
	Range	Median	<i>n</i>	Range	Median	<i>n</i>	Range	Median	<i>n</i>
Timothy	0.4–22.9	2.8	11	0.5–17.6	2.2	17	0.6–84.7	13.7*	28
Birch	0			0.35–45.9	5.7	17	0.5–134.3	20.1*	28
Alder	0			0.5–30.7	3.6	17	0.5–78.0	13.8*	27
Oak	0			0.35–21.2	1	15	0.35–37.1	6.5*	24
Mugwort	0			0			0.4–22.1	1.7	22
Dandelion	0			0			0.4–13.7	1.4	18
Marguerite	0			0			0.4–26.5	1.2	27
Grass-related food†									
Maize	0			0			0.35–2.4	1	8
Rice	0			0			0.4–2.6	0.9	8
Soya bean	0			0.6		1	0.4–11.8	0.8	9
Tomato	0			0.5–3.1	1	3	0.4–6.6	1.4	14
Wheat	1.8		1	0.6; 1.1		2	0.4–3.3	0.9	11
Tree-related food†									
Cashew nut	0			0			1.2–7.1	4.1	4
Hazel nut	0			0.5–2.4	1.1	5	0.35–6.3	1.9	18
Strawberry	0			0.7–3.2	0.7	4	0.5–3.4	1.5	14
Peanut	0			0.9; 1.9		2	0.4–147.1	1.3	19
Kiwi	0			0			0.6–2.2	0.7	5
Cherry	0			0			0.5; 0.6		2
Carrot	0			0.4–0.7	0.5	3	0.5–15.4	0.9	19
Brazil nut	0			0.9		1	0.6–20.2	0.9	6
Peach	0			0.5		1	0.4–1.1	0.8	7
Pistachio	0			0.4; 0.6		2	0.4–12.0	0.9	12
Plum	0			0.5		1	0.35–0.9	0.5	7
Potato	0			0.4–7.0	2.9	3	0.4–2.4	1.2	12
Apple	0			0.5–13.4	1.9	11	0.6–34.0	3.1	22
Compositae-related food†									
Banana	0			0.99		1	0.4–3.4	0.7	12
Onion	0			0			0.5–1.9	1.2	12
Sweet pepper	0			0			0.4–1.4	0.5	10
Lettuce	0			0			0.4–2.6	1	9
Anise	0			0			0.4–3.2	1.1	10
Fennel seed	0			0			0.4–2.6	0.8	13
Nonpollen-related allergens									
Avocado	0			0			0.4–1.0	0.6	6
Latex	0			0			0.35–1.5	0.5	9
Shrimp	2.3		1	0.5; 4.0		2	0.4–15.4	1.5	8
Animal mix	0.7–7.1	3.6	4	0.4–8.3	2.3	12	0.3–16.9	2.2	24
House dust mite	1.3; 2.0		2	0.5–10.4	1	5	0.5–6.3	1.8	10
Mould mix	0			1.9–8.7	5.8	4	0.5–4.0	1.2	4

* $P < 0.05$.

† Relations between pollen and food allergens according to Refs (16, 23, 38–40).

and/or marguerite and/or dandelion) (pan-pollen group). Two individuals (4%) were found sensitized only to grass and Compositae pollen. Patients belonging to the pan-pollen group had significantly higher IgE antibody levels to timothy grass pollen (median levels 13.7 kU_A/l) than patients belonging to the grass pollen and grass-tree pollen groups (2.8 and 2.2 kU_A/l, respectively) ($P = 0.005$) (Table 1). The pan-pollen group also demonstrated significantly higher IgE levels against birch, alder and oak compared with the grass-tree pollen group. For the two patients sensitized exclusively to grass and

Compositae, the IgE antibody levels to timothy grass pollen were 16.7 and 28.3 kU_A/l, respectively.

Sensitization profiles to single allergen components

The *Phleum* allergens Phl p 1, 4 and 5 were recognized by > 50% of the patients (Table 2). The median IgE antibody levels to nPhl p 4 were significantly higher in the pan-pollen group compared with the other two groups ($P < 0.05$). Nearly all patients in the two tree-related groups (43/45) had IgE against Bet v 1. However,

Table 2. Comparison of three groups of pollen-allergic-patients and their pattern of sensitization to both recombinant and native grass- and birch-pollen allergens, expressed as range and median of specific IgE-values (kU_A/l) for each allergen

Allergen	Grass (<i>n</i> = 11)				Grass-tree (<i>n</i> = 17)				Pan-pollen group (<i>n</i> = 28)			
	Range	Median	<i>n</i>	%	Range	Median	<i>n</i>	%	Range	Median	<i>n</i>	%
rPhl p 1	0.4–8.7	1.1	8	73	0.5–10.4	1.3	13	76	0.5–65.6	4.7	26	93
rPhl p 2	0.6; 0.7		2	18	0.3–3.5	0.5	7	41	0.6–15.0	2.6	10	36
nPhl p 4	0.5–1.8	1.4	6	55	0.4–7.0	0.8	11	65	0.4–27.4	3.9*	25	89
rPhl p 5	0.5–10.6	2.2	7	64	0.4–7.9	1.1	6	35	0.5–39.6	7.6	20	71
rPhl p 6	0.6–4.0	1.9	3	27	0.6–3.1	1.0	4	24	0.4–10.0	3.5	16	57
rPhl p 7	0	0	0		0	0	0		0	0	0	
rPhl p 11	0.5–2.3	0.7	4	36	0.5–7.4	2.4	5	29	0.4–14.2	1.2	12	43
rPhl p 12	0	0	0		0	0	0		1.1–3.1	1.8	4	14
rBet v 1	0		0		0.5–51.1	6.0	17	100	0.4–139.5	23.7	26	93
rBet v 2	0		0		0		0		1.4–3.8	3.2	4	14
rBet v 4	0		0		0		0		0	0	0	

* Comparison between the pan-pollen group and the grass and grass-tree groups; $P < 0.05$.

only four patients displayed IgE reactivity to profilin (Phl p 12 and Bet v 2) and they belonged to the pan-pollen group.

Pollen exposure

Evaluation of pollen exposure in Stockholm during 1988–2003 revealed that the total load of birch pollen was more than seven times higher than that of grass pollen (142 000 vs 20 200-pollen counts/m³). However, the total load of mugwort pollen was more than six times lower than that of grass pollen (3200 vs 20 200-pollen grains/m³).

IgE-reactivity to pollen-related food allergens

Among patients solely sensitized to grass pollen (*n* = 11), only one patient revealed IgE to pollen-related food allergens compared with 11 in the grass-tree pollen group (*n* = 17) and 22 in the pan-pollen group (*n* = 28) (Table 1). The number of positive IgE results to plant foods were significantly higher in the pan-pollen group compared with the grass-tree pollen group and grass pollen group (269 vs 40 and 1) ($P < 0.0001$). Although the pan-pollen group exhibited higher levels of allergen-specific IgE to several of the food allergens compared with the other two groups (Table 1), the difference was not significant. The two patients sensitized solely to grass and compositae exhibited three positive IgE antibody results to plant food.

Clinical reactivity to pollen-related food allergens

The number of patients stating any allergic symptom in response to plant food was significantly higher in the pan-pollen group than in the grass group ($P = 0.017$) (Table 3). The symptom most frequently reported was OAS, which was significantly more common in the pan-pollen group than the grass pollen group ($P = 0.007$).

Table 3. Comparison of the number of patients reporting occurrence of symptoms after intake of pollen-related food and types of symptoms in three different grass pollen-groups

	Grass (<i>n</i> = 11)		Grass-tree (<i>n</i> = 17)		Pan-pollen (<i>n</i> = 28)	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Any symptom	3*	27	11	65	21*	75
OAS	2*	18	10	59	20*	71
Asthma	1	9	2	12	5	18
Rhinoconjunctivitis	0	0	1	6	6	21
Oedema	0	0	3	18	6	21
Urticaria	0	0	2	12	5	18
Eczema	0	0	2	12	3	11

* Comparison between the grass group and pan-pollen group; $P < 0.05$.

In the grass-tree pollen group as well as in the pan-allergen group, hazelnut, apple, tomato, carrot and peanut were the allergens most often reported to elicit symptoms. The percentage of patients with allergen-specific IgE and a corresponding reported history was in both groups highest for apple, and in the pan-pollen group also for hazelnut, carrot and peanut. The four patients who revealed IgE antibodies to grass profilin reported symptoms to grass-, tree- and Compositae-related food allergens. All patients who reported symptoms associated to food-allergen intake had IgE-reactivity against Bet v 1. Neither of the two individuals sensitized only to grass and Compositae pollen reported symptoms after intake of plant food.

Comparison of food-specific IgE and clinical history

The concordance and discordance between the results concerning IgE antibodies to food and the information obtained by questionnaire was compared. The pan-pollen group showed a significantly lower mean concordance

Table 4. Comparison between answers/information obtained by questionnaire (Q) and results of specific IgE for patients sensitized to grass-tree pollen (A) and grass, tree and Compositae pollen (B)

Allergen	Concordance			Discordance		
	Q+/IgE+	Q-/IgE-	Total (%)	Q+/IgE-	Q-/IgE+	Total (%)
(A) Grass-tree pollen group*						
Rice	0	11	11 (100)	0	0	0 (0)
Soya bean	0	10	10 (91)	1	0	1 (9)
Tomato	1	5	6 (55)	3	2	5 (45)
Wheat	0	10	10 (91)	0	1	1 (9)
Hazelnut	1	0	1 (9)	7	3	10 (91)
Strawberry	0	7	7 (64)	1	3	4 (36)
Peanut	1	9	10 (91)	1	0	1 (9)
Carrot	1	7	8 (73)	2	1	3 (27)
Potato	0	8	8 (73)	1	2	3 (27)
Apple	6	1	7 (64)	1	3	4 (36)
Onion	0	10	10 (91)	1	0	1 (9)
Egg	1	10	11 (100)	0	0	0 (0)
Fish	0	10	10 (91)	1	0	1 (9)
Total	11	98	109	19	15	34
(B) Pan-pollen group†						
Rice	0	15	15 (71)	0	6	6 (29)
Soya bean	3	13	16 (76)	1	4	5 (24)
Tomato	5	4	9 (43)	6	6	12 (57)
Wheat	1	11	12 (57)	2	7	9 (43)
Hazelnut	14	2	16 (76)	3	2	5 (24)
Strawberry	3	6	9 (43)	3	9	12 (57)
Peanut	10	4	14 (67)	1	6	7 (33)
Carrot	10	2	12 (57)	2	7	9 (43)
Potato	2	7	9 (43)	4	8	12 (57)
Apple	16	1	17 (81)	1	3	4 (19)
Onion	0	9	9 (43)	2	10	12 (57)
Egg	0	19	19 (90)	2	0	2 (10)
Fish	1	19	20 (95)	1	0	1 (5)
Total	65	112	177	28	68	96

* Mean concordance 76%; mean discordance 24%.

† Mean concordance 65%; mean discordance 35%.

for all the different allergens taken together than the grass-tree pollen group (65% vs 76%) ($P = 0.018$) (Table 4A,B), although no statistically significant differences could be found for the individual allergens.

Discussion

In the present study, we have analysed the IgE-reactivity profile among grass pollen-allergic patients from northern Europe and compared their results with self-reported reactions to pollen-related food allergens. An in-depth *in vitro* analysis was performed with 10 respiratory and 26 food allergen extracts. The results revealed that only a minority of grass pollen-allergic patients was solely sensitized to grass pollen (19%). The majority (78%) reacted also to birch. When we analysed the allergen profile in greater detail with a panel of recombinant and native pollen allergens, we found that the dominating *Phleum* allergens were Phl p 1, 4 and 5, as has been

reported in earlier studies (6, 29). Phl p 2 on the other hand was of minor importance. This allergen has previously been documented to be a major allergen in central and southern Europe (2, 5, 6, 30). The discrepancy is probably related to geographical variations in allergen exposure. Nearly all subjects in the grass and tree and pan-pollen groups reacted to the major birch pollen allergen Bet v 1, which is most likely due to the high birch pollen exposure in Sweden (31). The median levels of IgE to Bet v 1 were also higher than the median levels against any of different grass pollen allergens. In contrast to other studies, we report only four individuals with IgE reactivity to profilin (Phl p 12 and Bet v 2). These individuals were all found in the pan-pollen group. Our finding is quite the opposite to previous studies that demonstrated an IgE binding frequency to Phl p 12 of 15–71% among patients allergic to grass pollen (5, 6) or to birch, grass and/or mugwort pollen (32). Thus, while sensitization to profilin is abundant in central and southern Europe (33), it seems to be rare in northern Europe. Furthermore, Bet v 1 is present in high amounts in birch pollen (34) which may be the reason why birch-pollen-sensitized patients in Sweden have IgE for Bet v 1 rather than for profilin (31). However, the clinical relevance of profilin is still under debate (32).

Interestingly, patients in the grass pollen group showed almost no IgE reactivity to plant food, whereas such reactivity was noted in the grass-tree pollen group. This tendency was even more pronounced in the pan-pollen group, where the patients significantly more often showed IgE responses to pollen-associated food allergens than did the two other groups, in agreement with other studies (8). Patients in the pan-pollen group were also more prone to mount higher IgE levels in response to both grass- and tree-related food allergens than patients in the grass- and grass-tree pollen groups. We could not explain the general trend of more sensitization in the pan-pollen group with an over-representation of highly atopic patients in this group, as we did not see higher IgE antibody levels or an increased number of positive tests to the group of nonpollen-related allergens. New, as yet undescribed cross-reactive structures could be involved.

Nearly 75% of the grass pollen-allergic patients also reacted to Bet v 1. This may explain why the majority of reported symptoms with corresponding IgE-reactivity were associated to birch pollen-related food allergens. Several molecules that are homologous to Bet v 1 are related with the clustered reactivity of birch/alder/oak (35). These allergens can cause food-related allergic disorders and have been identified in plant food (Mal d 1 a in apple, Pru av 1 in cherry, Dau c 1 in carrot, and Api g 1 in celery) (36). As birch pollen-associated food allergens often are unstable proteins, the consequences of ingestion of these allergenic foods are generally undramatic and in the majority of cases limited to OAS, as was the case in our study, although severe local reactions have also been reported (37).

The highest frequency of self-reported symptoms in this study was seen among patients in the pan-pollen group. However, comparison between responses obtained by the questionnaire and the IgE reactivity to the pollen-related food allergens revealed that the overall mean concordance was significantly lower for the pan-pollen group than for the grass-tree pollen group. Diagnostic tests based on recombinant marker allergens will be useful in the understanding of cross-reactivity between pollen and food allergens. Furthermore, recombinant nonglycosylated allergens could be a way to overcome the CCD reactivity and to obtain an *in vitro* diagnosis closer to the clinical reactivity. Such a molecule-based approach will not only enhance the diagnosis but also help to select optimal therapy strategies.

In summary, we have shown that sensitization solely to grass pollen is rare among grass pollen-allergic patients from northern Europe. The majorities are also sensitized to birch because of the heavy birch pollen exposure in this area. The detailed analysis of the reactivity profile performed with native and recombinant allergens

revealed that Bet v 1 accounts for a high percentage of pollen-specific IgE and that profilin is of minor importance. Patients sensitized to grass pollen only showed very few reactions to plant food, in contrast to patients sensitized to birch, grass and Compositae. The study emphasizes the need for better understanding of the sensitization profiles as well as well-defined allergen molecules in the analysis of cross-reactivity between pollen and food allergens.

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