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ORIGINAL RESEARCH

Sensitization Profiles of Timothy Grass Pollen in Northern China

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Purpose: Grass pollen is an important cause of IgE-mediated allergy in countries worldwide, especially within Europe. However, there has been no research on grass pollen allergy in northern China. We aimed to determine the status of grass pollen allergy and the sensitization patterns to *Phleum pratense* (*P. pratense*) in northern China.

Patients and Methods: Pollen data were collected for three geographic areas (Beijing, Shenmu, Shizuishan) in northern China. The study enrolled 101 patients (62 men; age range, 1–64 years; median age, 10 years) who had allergic rhinoconjunctivitis and/or asthma during the grass pollen season and positive skin prick test results positive to *P. pratense*. Serum-specific IgE (sIgE) against Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p 12 was measured by ImmunoCAP.

Results: The pollen season of *P. pratense* was from June to September in Beijing, May to September in Shenmu and July to August in Shizuishan. *P. pratense* pollen accounted for 2–3% of the annual pollen index of total pollen counts. Among 101 patients with positive skin prick test results to *P. pratense*, 72% had detectable sIgE to *P. pratense*. Phl p 12 was the most frequently recognized component (45%), followed by Phl p 1 (22%), Phl p 5 (14%), Phl p 6 (8%) and Phl p 7 (3%). No patients had sIgE to Phl p 2. Ten sensitization patterns to the six components were observed. High rate of sIgE to Phl p 12 was positively correlated with co-sensitization to weed or tree pollen.

Conclusion: Considering the pollen concentration, *P. pratense* was a minor pollen allergen in northern China and its pollen season overlapped with that of weed pollen. IgE sensitization to *P. pratense* was likely to be induced by cross-reactivity between grass pollen allergy and weed/tree pollen allergy.

Keywords: allergic rhinitis, allergen components, grass pollen allergy, *Phleum pratense*, Phl p 12

Introduction

The term "grass" refers to plants belonging to the Poaceae family. However, "grass" is often confused with "weed" in China. Even in some literature reports, grass was misrepresented as mugwort, Japanese hop or other weed species. *Poaceae* pollen is the leading cause of pollen allergy in countries worldwide, especially in Europe and north America.[1] During the *Poaceae* pollen season, generally late spring and early summer in the Northern Hemisphere, grasses produce large quantities of pollen and may cause allergic rhinoconjunctivitis (ARC) or asthma in susceptible individuals. It was estimated that 15% of the general population in the United States and 20% of the general population in Europe suffer from grass pollen allergy.[2,3]

Three Poaceae subfamilies: Pooideae, Chloridoideae and Panicoideae, account for >95% of allergy-relevant grass species.[4] The allergenicity of different species in the Poaceae family shows extensive cross-reactivity.[5,6] For example, determination

of IgE to *Phleum pratense* (*P. pratense*, timothy grass), the most common pollen for grass allergy, produced similar results in the majority of species in the Poaceae family.[7] To date, nine allergens from *P. pratense* have been officially recorded by the IUIS Allergen Nomenclature Sub-Committee. Among them, the combination of Phl p 1 and Phl p 5 is a unique marker for timothy grass sensitization, while Phl p 7 (polcalcin) and Phl p 12 (profilin) have broad cross-reactivity. Phl p 1 shows 80% homology to group 1 allergens from other grasses[8] and shares IgE epitopes with eight grass pollens.[9] Phl p 5 shows high cross-reactivity with other group 5 allergens in the family.[4,10] Therefore, we chose *P. pratense* as a representative allergy relevant grass pollen.

Data on sensitization profiles to grass pollen are available in Europe and the United States but are relatively infrequent elsewhere. Only a few studies on grass pollen allergy in Asia and Africa have been published.[11–13]

China has a vast territory that crosses approximately 50 latitudes. Thus, the vegetation from the north to the south of China shows large diversity.[14,15] In southern China, taking Zhongshan city in Guangzhou province as an example, *Poaceae* is the most abundant pollen throughout the year, with a peak season in November and accounts for 27% of the annual pollen quantity.[16] Following *Poaceae, Pinus, Taxodiaceae, Cupressaceae, Broussonetia* and *Casuarinaceae* are the other common pollen types with peak seasons in February and March.[16] In northern China, trees and weeds produce the major pollen types, and format two main allergenic pollen seasons.[17,18] One season occurs in spring and is caused by tree pollens including *Salix caprea, Sabina chinensis, Fraxinus americana, Platanus acerifolia, Betula*, and *Populus*. And the other occurs in late summer and autumn and is caused by weed pollens, such as *Artemisia, Amaranthus retroflexus, Humulus scandens, Kochia scoparia* and *Chenopodium glaucum*. However, sensitization to grass pollen has never been assessed. In this multicenter study, to determine the impact of pollen exposure in terms of duration and concentration, we analyzed the IgE sensitization profiles to *P. pratense* allergens in patients from northern China.

Materials and Methods
Ethics Statement
This study has been performed in accordance with the principles stated in the Declaration of Helsinki, and the study protocol was approved by the Research and Ethics Board of Peking Union Medical College Hospital (approval number: ZS-2342). Informed consent was signed by all patients or their parents before participation.

Patients and Study Design
This was a cross-sectional study on patients with seasonal ARC and/or asthma during the grass pollen season who visited the outpatient department for allergy in three cities in China. A total of 101 patients (62 men) were recruited from June to September 2020. Among them, 51 were from greater Beijing (BJ) including Beijing city and its surrounding area, 37 were from Shenmu city (SM) in Shanxi province, and 13 were from Shizuishan city (SZS) in Ningxia Hui Autonomous Region. The patients ranged in age from 1 to 64 years, with a median age of 10 years. According to the standard protocol, all patients underwent a comprehensive diagnostic workup including questionnaire completion, physical examination and skin prick test (SPT). Inclusion criteria were self-reported symptoms of ARC and/or asthma during the grass pollen season for at least 2 years, and positive SPT results to timothy grass. Exclusion criteria were ongoing or complete allergen-specific immunotherapy with pollen, and/or the presence of perennial symptoms of ARC or asthma. The study design is illustrated in Figure 1.

Pollen Concentration
We retrospectively analyzed the pollen data in BJ, SM and SZS to focus on two geographic areas: northern and northwest China. Daily pollen monitoring was performed from 01 January to 31 December 2013 in the three cities and from May to October 2020 in SM and March to October 2020 in SZS, by a gravitational method using a modified Durham pollen sampler described by Ye[19] and white petrolatum as an adhesive. The pollen samplers for BJ, SZS and SM were set on the top of buildings located in Peking Union Medical College Hospital, The No.1 People's Hospital of Shizuishan and Shenmu Hospital, respectively, at a height of 16–18 m above the ground and with good ventilation. Each slide was placed daily at 08:00 am and collected 24 hours later. The daily slides were attained, identified and counted by a trained technician. The daily pollen concentrations were reported as grains/m^3. For ease of description, we used monthly and annual pollen concentrations for this study, presented as grains/m^3/month and grains/m^3/year
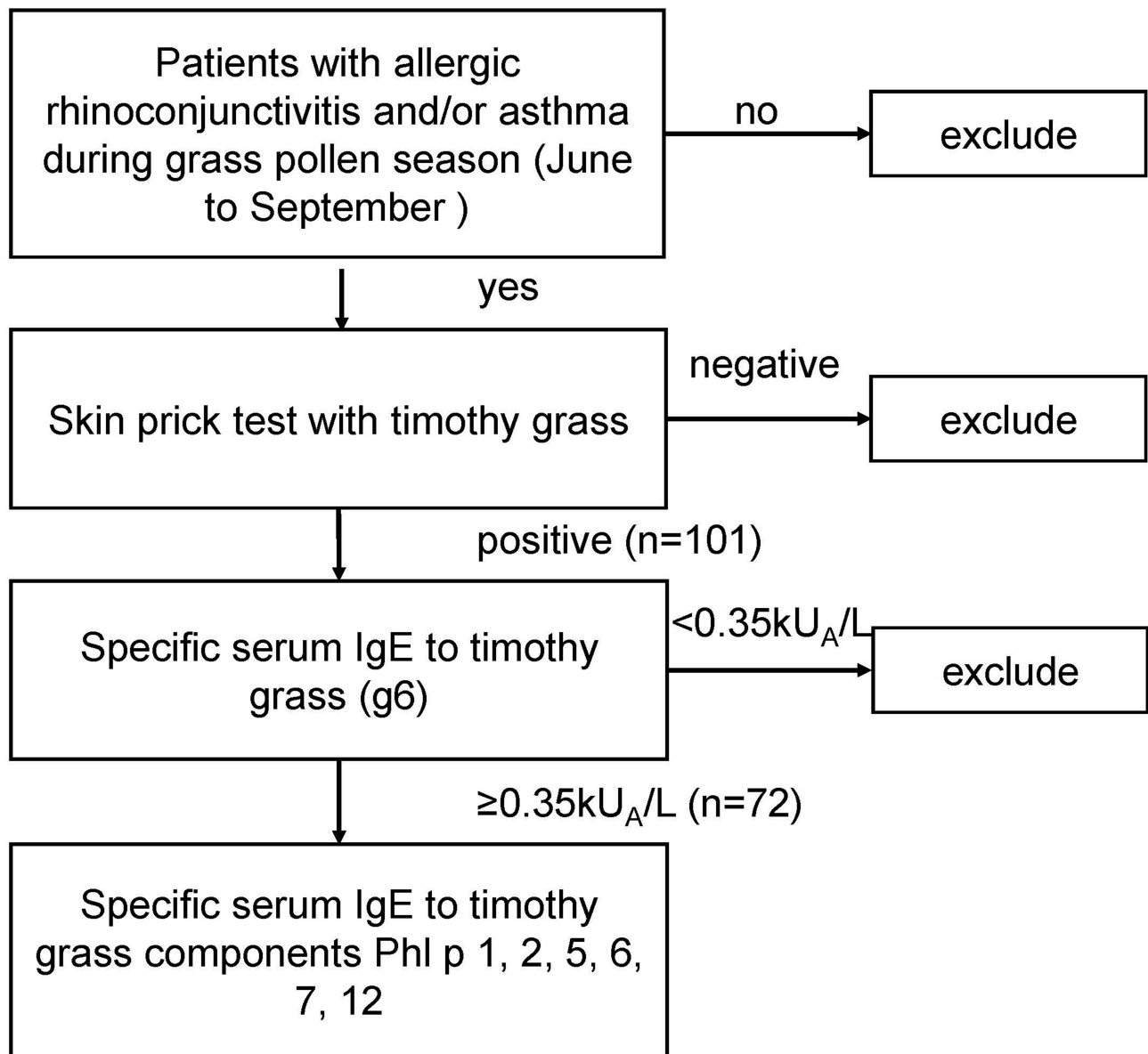
Figure 1 Study design.

respectively. Thirty-eight pollen species from weeds, trees and grasses were counted. The duration of each pollen season was calculated based on the EAACI definition.[20] The definition of the birch pollen season was used to calculate the pollen season for trees and weeds pollen.

Skin Prick Test

An SPT with timothy grass extract (Soluprick SQ *Phleum pratense*, ALK, Denmark) was performed in all patients. Common allergic pollen including *Salix caprea, Sabina chinensis, Fraxinus americana, Platanus acerifolia, Betula, Populus, Artemisia, Amaranthus retroflexus,*

Humulus scandens and *Chenopodium glaucum* was also evaluated by SPT. A positive reaction was defined as mean wheal diameter $\geq$ 3 mm.

Serum Specific IgE Measurement

Serum-specific IgE (sIgE) against timothy grass (g6), its components Phl p 1 (g205), Phl p 2 (g206), Phl p 5 (g215), Phl p 6 (g209), Phl p 7 (g210), and Phl p 12 (g212), and mugwort (w6) were tested by the ImmunoCAP allergen system (Phadia 1000, ThermoFisher Scientific, Uppsala, Sweden). A positive value was defined as detectable sIgE $\geq$ $0.35kU_A/L$.

Statistical Analysis

Statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Differences in the prevalence of timothy grass components in the three areas were analyzed by the Fisher test. The Spearman correlation test was used to analyze the potential correlations between different components of grass pollen and co-sensitization profiles. P values of p<0.05 were considered to indicate statistical significance.

Results

Pollen Data

The daily and monthly pollen concentrations in BJ, SM and SZS are shown in Figure 2A and B. Two predominant pollen seasons were observed. One season was caused by tree pollens from March to May, with an annual pollen index (API) of 9614 grains/m^3/year in BJ, 4018 grains/m^3/year in SM and 3133 grains/m^3/year in SZS. The other season occurred from July to September or even October and was caused by *Artemisia* (mugwort) and other weed pollens, such as

Humulus, Chenopodium, and *Amaranthaceae*. The API of the weed pollen season was 19,400 grains/m^3/year in BJ, 23,262 grains/m^3/year in SM and 18,959 grains/m^3/year in SZS (Figure 2C). In terms of individual species, mugwort had the highest annual sums of recorded counts (Figure 2C). Specifically, the API of mugwort was 10,228 grains/m^3/year in BJ, 22,230 grains/m^3/year in SM and 7354 grains/m^3/year in SZS.

The API of *Poaceae* pollen was 708 grains/m^3/year in BJ with a pollen season from June to September, 798 grains/m^3/year in SM with a pollen season from May to September and 420 grains/m^3/year in SZS with the pollen season from July to August.

Demographic and Clinical Characteristics of the Patients

Based on the inclusion criteria, 101 patients were recruited (62 men, 39 women) with a median age of 10 years (rang, 1–64 years). Sixty-three (62%) patients were children aged ≤ 13 years. The age distribution of the patients is illustrated in
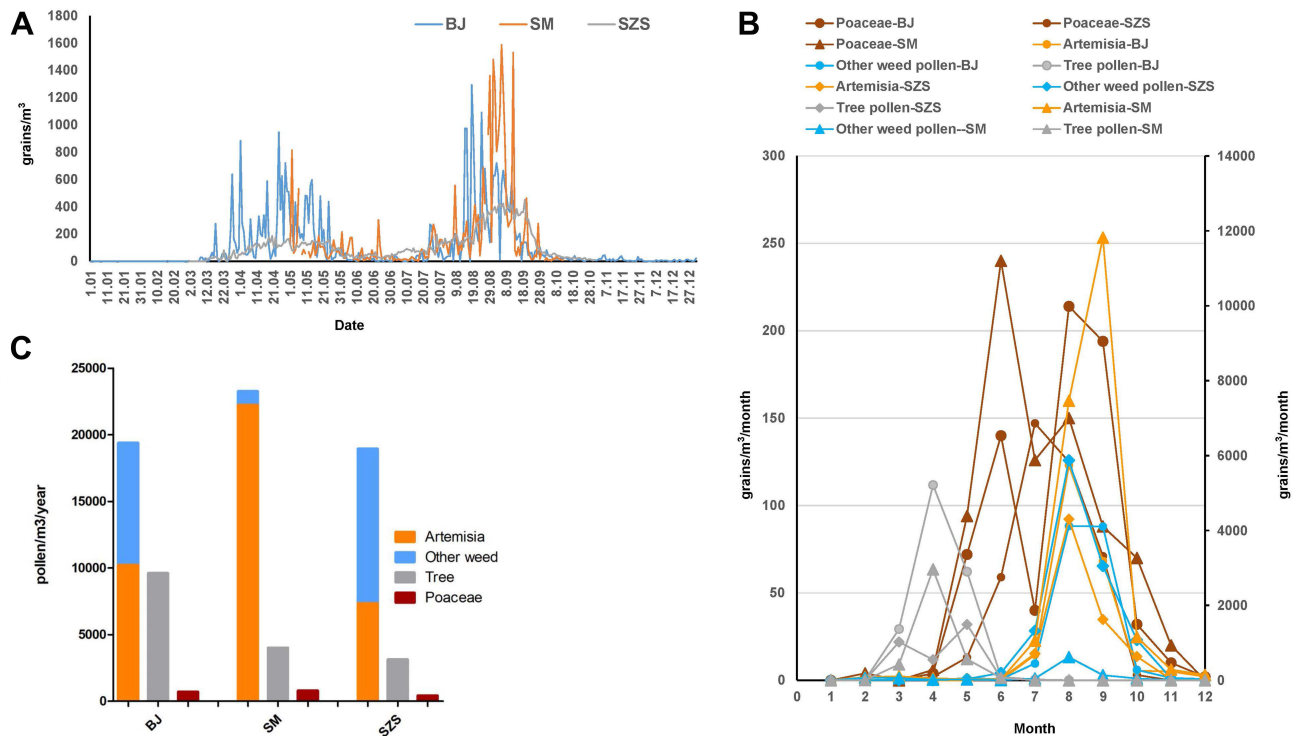

Figure 2 Pollen concentration in three areas. Pollen monitoring was performed by a gravitational method using a modified Durham pollen sampler described by Ye.[19]
Notes: (**A**) Daily pollen concentration in BJ (data collected from 01 January to 31 December, 2013), SM (data collected from 01 May to 12 October, 2020) and SZS (data collected from 01 March to 31 October, 2020). (**B**) Monthly pollen concentration in BJ, SM and SZS. Pollen monitoring was performed from 01 January to 31 December 2013. The concentration of *Poaceae* pollen refers to the left vertical axis and the concentration of *Artemisia*, other weed and tree pollens refer to the right vertical axis. The season of *Poaceae* pollen was from June to September or even October, and overlapped with weed pollen. The *Poaceae* pollen concentration is much lower than the other three types of pollen. (**C**) The annual pollen index (API) of *Poaceae, Artemisia*, other weed and tree pollens. *Artemisia* is the major type in weed pollens. Compared to the other three types pollen, the API of *Poaceae* pollen is much lower.
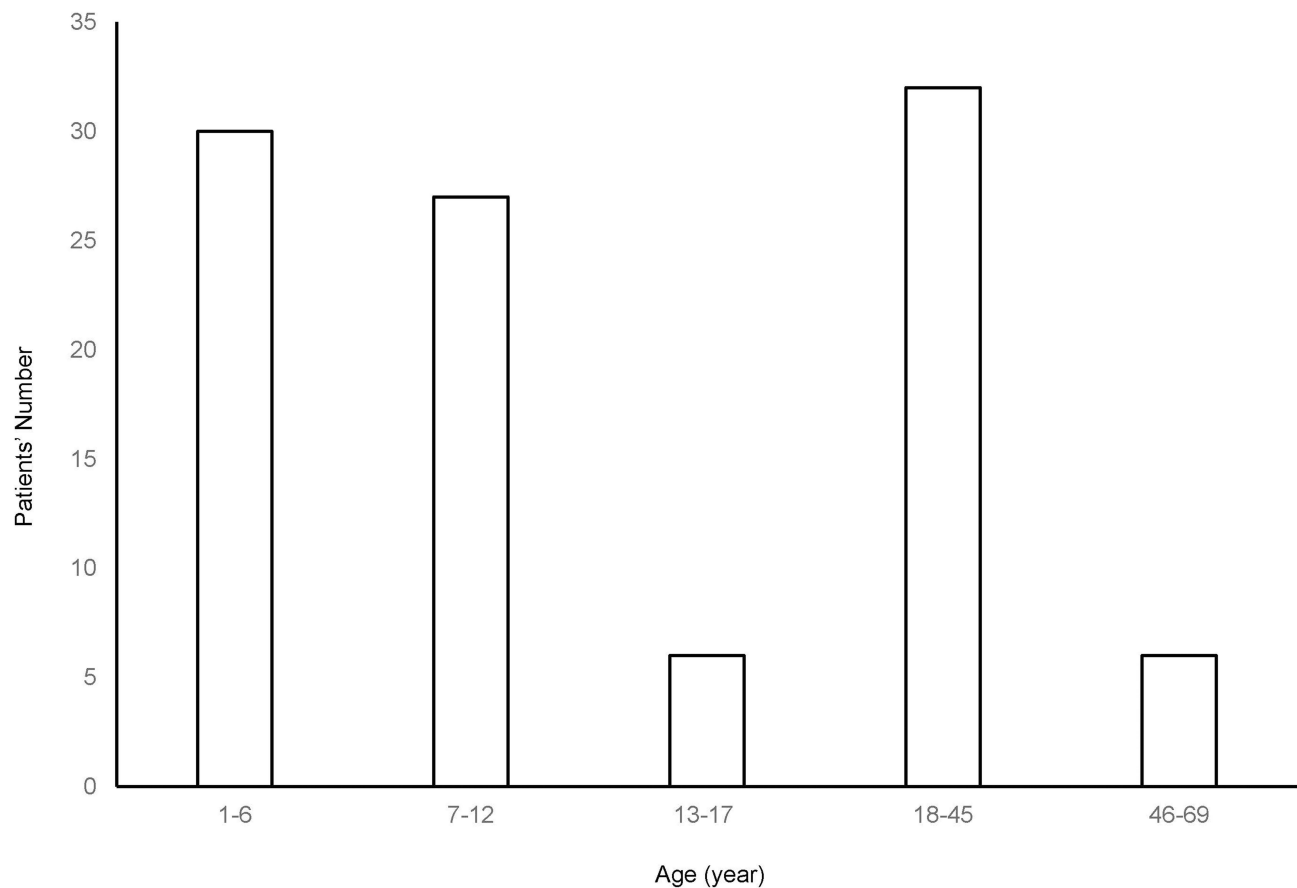
Figure 3 The age distribution of patients.
Notes: 29% of patients were aged 1–6 years, 26% aged 7–12 years and 31% aged 18–45 years.

Figure 3. All 101 of patients presented with ARC, and 73 patients (72%) had ARC combined with asthma.

IgE Reactivity Profiles to Timothy Grass and Its Components

Timothy grass sIgE was detected in 73 (72%) of 101 patients with positive SPT results to timothy grass. Timothy grass allergen components were further assessed in these 73 patients. Phl p 12 was the most frequently recognized allergen with a prevalence of 45% (n=33/73), followed by Phl p 1 (22%; n=16/73), Phl p 5 (14%, n=10/73), Phl p 6 (8%, n=6/73) and Phl p 7 (3%, n=2/73). No patients were positive for Phl p 2. The positive rates for each component are summarized in Table 1. Ten patterns of sIgE against the timothy grass components were observed (Table 2).

The prevalence of sensitization to the components was quite different among the three areas. The frequency of sIgE to Phl p 1, Phl p 5 and Phl p 6 in SM were 63%, 63% and 38%, respectively, and much higher than the

corresponding frequency in BJ (4%, 0% and 0%; p<0.05) and SZS (25%, 0% and 0%; p<0.05). The prevalence of sIgE to Phl p 12 in BJ (49%) and SZS (75%) was significantly higher than that in SM (19%; p<0.05).

Co-Sensitization Profiles

On the basis of their reactivity to common weed and tree pollens, timothy grass-sensitized patients were assigned to timothy grass monosensitization and polysensitization groups. In the 73 patients with both positive SPT results and sIgE to timothy grass, only 5 (7%) were monosensitized and 68 (93%) were polysensitized to other pollen. Meanwhile, in the 28 patients with positive SPT results but no sIgE to timothy grass, 10 (36%) had no detectable sIgE to the common pollen allergens. For the polysensitized patients, 65 (89%) patients were positive to weed pollen and 63 (86%) positive to mugwort pollen, while 65 (89%) were positive to tree pollen and 45 (62%) were sensitized to birch pollen.

Table 1 Prevalence of *Phleum pratense* Pollen Components

	Positive Rate of Specific IgE to Each Component of *Phleum pratense* in Different Areas							
	Total (n=73)	Prevalence	Beijing (n=49)	Prevalence	Shenmu (n=16)	Prevalence	Shizuishan (n=13)	Prevalence
Phl p 1	16	22%	4	8%	10	63%	2	25%
Phl p 2	0	0%	0	0%	0	0%	0	0%
Phl p 5	10	14%	0	0%	10	63%	0	0%
Phl p 6	6	8%	0	0%	6	38%	0	0%
Phl p 7	2	3%	2	4%	0	0%	0	0%
Phl p 12	33	45%	24	49%	3	19%	6	75%

Table 2 Number of Patients in Relation to IgE Sensitization Pattern to Each Component of *Phleum pratense*

Phl p 1	Phl p 2	Phl p 5	Phl p 6	Phl p 7	Phl p 12	Number
−	−	−	−	−	+	28
−	−	−	−	−	−	24
+	−	−	−	−	+	3
−	−	+	+	−	−	4
+	−	−	−	−	−	7
+	−	+	+	−	−	3
+	−	+	−	−	−	3
−	−	−	+	−	+	1
−	−	−	−	+	−	1
−	−	−	−	+	+	1

In the timothy grass polysensitization group, the correlations between sIgE to timothy grass components and co-sensitization profiles to weed or tree pollen were analyzed. The presence of Phl p 1, Phl p 5 and/or Phl p 6 was considered a genuine grass allergy. Significant negative correlations between genuine grass allergy and sensitization to weed pollen (R = -0.356, p <0.05) or tree pollen (R = -0.337, p <0.05) were found. Phl p 12 had a weak positive association with sensitization to weed pollen (R = 0.267, p <0.05) or tree pollen (R = 0.271, p < 0.05).

Discussion

Grasses are wildly grown in northern China for multiple purposes, such as cattle feeding, or reinforcement and improvement of soil and city lawns. *Leymus chinensis, Dactylis glomerata* and *Bromus inermis Leyss* belonging to the Pooideae subfamily are the most commonly grown species. In the present study, we collected pollen data for three cities located in three geographic areas in northern China. The results showed that the pollen season of *Pooideae* species lasted for 5–6 months, from May to September or even October. The season started at the end of the tree pollen season, but overlapped with the weed pollen season in August and September. June was relatively pure for the grass pollen season with no overlap for the tree and weed pollen seasons. The pollen season duration was similar among the three areas. Concerning the pollen quantities, mugwort pollen was predominant in August and September. Specifically, mugwort pollen accounted for 79% of the annual sum of pollen counts in SM, 34% in BJ and 33% in SZS. Pollen from other weed species, mainly *Chenopodium, Humulus hop*, and *Amaranthaceae*, were also important allergens in BJ (31% of the annual sum of pollen counts) and SZS (52%). Compared with these major pollens, the API of grass was very low, accounting for 2–3% of the annual sum of pollen counts.

Based on the large degree of cross-reactivity between timothy grass and other grass species,[9] we used timothy grass as representative grass species. Phl p 1 is the most prevalent component in patients with grass allergy and is a major allergen in grass pollen. Phl p 5 and Phl p 6 are other major allergens in grass pollen with prevalence of more than 50%.[21] Phl p 1 is present in all types of grass pollen, while Phl p 5 and Phl p 6 are specific for *Pooideae* pollen. Thus, positive results to Phl p 1 and Phl p 5 are considered to be genuine grass allergy. In Europe, more than 90% of grass allergy patients were sensitized to Phl p 1, of whom 20% were monosensitized to Phl p 1.[21–23] Following Phl p 1, the prevalence of sensitization to Phl p 2, Phl p 5 and Phl p 6 was around 50% for each component. In the present study, the findings for patients in China were quite different from those for patients in Europe. In China, only 22% of patients were sensitized to Phl p 1, of whom 43% (n=7/16) were monosensitized to Phl p 1. The positive rate of sensitization to Phl p 2 (0%), Phl p 5 (14%) and Phl p 6 (8%) in China were significantly lower than those in Europe. A study on grass sensitization in southern China showed similar, low prevalences of

sensitization to Phl p 1 (22% vs 17%), Phl p 5 (14% vs 8%) and Phl p 6 (8% vs 8%), but found no patients who were monosensitized to Phl p 1.[24] The difference in sensitization patterns to grass pollen components could be essential when component-resolved immunotherapy is considered for patients with grass allergy. BM32 is a novel recombinant vaccine for grass pollen allergy that consists of hypoallergenic peptides from Phl p 1, Phl p 2, Phl p 5 and Phl p 6.[25] The vaccine was designed on the basis of allergenic relevance and the prevalence of sensitization to grass components in the population.[26] However, the sensitization patterns were highly heterogeneous and showed large variation among different geographic areas. In our study population, no patients had sIgE to Phl p 2. Therefore, component-based immunotherapy vaccines should consider the heterogeneity and local characteristics of sensitization patterns for tailoring to patients.

Phl p 7, a calcium-binding protein and Phl p 12, a pan-allergen profilin, are the main cross-reactive components between grass, tree and weed pollens. The positive rate of sensitization to Phl p 7 and Phl p 12 in Europe were lower than those to the major allergens mentioned above, varying from 2% to 5% for Phl p 7 and 2–25% for Phl p 12.[21,27] Similarly, in southern China, sensitization to Phl p 7 and Phl p 12 was detected in 8% of the patients with positive sIgE to timothy grass allergens.[24] The prevalence of sensitization to Phl p 7 was also low in the present study. In contrast to previous reports, we found that approximately one-half of the patients in this study were positive for sensitization to Phl p 12 and 85% (n=28/33) were monosensitized to Phl p 12. There was a positive correlation between Phl p 12 sensitization and weed/tree pollen sensitization. It is known that the prevalence of sensitization to pan-allergens is higher in patients from areas where pollen seasons overlap, especially when major allergens appear during the same period. Based on the pollen data, all three study areas had extensive exposure to weed pollen, particularly mugwort pollen. The API for weeds was approximately 30–40 times higher than that for grasses and the pollen seasons of weeds and grasses overlapped. Based on the sensitization data, 93% of timothy grass sensitized patients were co-sensitized to weed and/or tree pollen, and the majority of them had positive results to mugwort and/or birch pollen. All patients with sIgE to Phl p 12 had positive results to mugwort pollen. It has been demonstrated that Phl p 12 sIgE detection is positively correlated with Art v 4, a profilin allergen in mugwort pollen.[28] Thus, the high rate of sensitization to Phl p 12 was considered to result from cross-reactivity with weed/tree

pollen (especially mugwort) allergy. It was shown that cross-reactive carbohydrate determinants (CCDs) have a low influence on Phl p 12 sensitization.[28] In contrast, genuine allergy to timothy grass (positive results to Phl p 1, Phl p 5, and/or Phl p 6) was negatively correlated with weed or tree pollen sensitization.

There were some limitations to the present study. First, because grass pollen components have not been registered for clinical use in China and it is difficult to import them during the current COVID-19 pandemic, Phl p 4 was not detected. Phl p4 contains CCDs and shows broad cross-reactivity among grass pollens. Second, the pollen data is not comprehensive and satisfactory. Based on various reasons, integrated daily pollen concentration from 2020 in three study areas are lacking. To describe the API of different pollen species and the pollen season, pollen concentration from 2013 was used.

Conclusions

In summary, this study analyzed the sensitization profiles to timothy grass pollen in northern China. On the basis of the pollen concentration and sensitization patterns, timothy grass pollen was a minor allergic pollen in northern China, and approximately one-half of the sensitized patients were induced by weed and/or tree pollen allergy.

Abbreviations

ARC, allergic rhinoconjunctivitis; AIT, allergen-specific immunotherapy; API, annual pollen index; BJ, Beijing; CCD, cross-reactive carbohydrate determinants; SPT, skin prick test; SM, Shenmu; SZS, Shizuishan city; sIgE, specific IgE.

Data Sharing Statement

The data and materials are available from the corresponding authors based on reasonable requirement.

Ethics Approval and Informed Consent

This study has been performed in accordance with the principles stated in the Declaration of Helsinki, and the study protocol was approved by the Research and Ethics Board of Peking Union Medical College Hospital (approval number: ZS-2342).

Consent for Publication

All authors confirm that the details of the manuscript can be published.

Acknowledgments

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval for the version to be published; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors report no competing interests.

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