



Major grass pollen allergens and components detected in a southern Chinese cohort of patients with allergic rhinitis and/or asthma



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ABSTRACT

There is so far a paucity of data about allergen component-resolved diagnosis, and the prevalence of grass pollen allergen components in China, in contrast to those from western countries. Even in this country, allergies to grass pollen allergen components in the vast south are inadequately described. This study aimed to determine the major sensitizing grass pollens in Guangzhou, the largest city in Guangdong province of southern China. Included in this study were 258 patients having allergic rhinitis with or without asthma and 88 healthy controls. ImmunoCap100 was used to examine the serum samples for sIgE to Bermuda, Timothy, and *Humulus scandens*. Subjects who tested positive were further examined for sIgE to Bermuda antigen Cyn d 1, Timothy antigens Phl p 1/4/5/6/7/11/12, and CCD. The relationship of grass pollen allergy to specific antigen sensitization was assessed. As a result, 22.5% of patients with allergic rhinitis and/or asthma were positive for Bermuda-sIgE, 13.6% for Timothy-sIgE, and 7.0% for *Humulus scandens*-sIgE. These patients were more likely to be sensitized compared with controls ($P < 0.001$). Of the Bermuda-sIgE positive patients, 53.4% were Cyn d 1 positive and 60.3% were Timothy-sIgE positive. Of the Timothy positive patients, 100% were positive for Phl p 4, 17.1% were positive for Phl p 1 and 8.6% tested positive for Phl p 5/6/7/11/12. Patients with high Bermuda-sIgE levels were more likely to be positive for other grasses. In 41.4% of Bermuda grass positive patients, CCD-sIgE was also positive. Sensitization to Phl p 4 was significantly correlated with CCD ($r_s = 0.928$). In summary, we found that these southern Chinese patients with allergic rhinitis and/or asthma tested positive for Bermuda, Timothy, and *Humulus scandens* IgE. A high Bermuda-sIgE level may predict sensitization to other grasses. Correlations between sensitization to CCD and grass pollen allergens suggested a likelihood of cross-reactivity. Further in vitro inhibition assays are required to confirm this relationship.

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1. Introduction

Grasses produce large quantities of pollen during the pollen seasons around the world (D'Amato et al., 2007; Andersson and Lidholm, 2003; Suphioglu, 2000). Grass pollen as allergen is involved in 50% of IgE-related allergic diseases, and represents

an important cause of allergic respiratory diseases worldwide (D'Amato et al., 2007; Davies et al., 2012), in particular, allergic rhinitis and asthma (Walls et al., 2005; Pollart et al., 1988). Among the pollen-producing grasses, Poaceae family are the most common plants (Freye, 2008), especially the Poa genus, which includes species such as Timothy grass (*Phleum pratense*), rye grass (*Lolium* sp.), and cocksfoot (*Dactylis* sp.). Also implicated in allergic respiratory diseases have been the Bermuda grass (*Cynodon dactylon*) of Panicoideae family and the Japanese hop (*Humulus scandens*) of Cannabaceae family.

Although grasses present a worldwide coverage, the impact of different grass pollens on human allergy varies across geographical areas, generally known as regional specificity in pollen allergies. For example, epidemiologic studies have revealed that allergies to

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Timothy and Bermuda grasses mainly affect warm tropical and sub-tropical populations (Walls et al., 2005; Freye, 2008). In northern China and South Korea, Japanese hop (*Humulus scandens*) is a major grass pollen allergen during summer and autumn (Ouyang et al., 2012; Jeong et al., 2013). However, comparatively fewer cases of *Humulus scandens* allergy are reported in southern China.

The variation in pollen allergies due to geographic distribution has presented a number of challenges for the diagnosis and treatment. Previous studies on pollen allergens have shown a high prevalence of tree pollen allergy in Guangdong, the largest province of southern China (Xiaohong et al., 2013). This is likely due to the subtropical Guangdong climate that favors the growth of trees over grasses. As far as pollen allergy is concerned, southern Chinese showed a relatively low prevalence of allergy to grasses, which is drastically different from the findings in northern China. On the other hand, common allergenic grasses of northern China (such as Japanese hop, ragweed, and quinoa) are relatively scarce in Guangdong. It would also be possible that standard allergen test panels used to detect pollen allergies in China do not fully include a spectrum of Guangdong-specific pollens (Xiaohong et al., 2013). Owing to this limitation, few studies specifically looked at pollen allergens in Guangdong. Currently published data about China's grass pollen allergens were mainly focused on regions of continental China north to Yangtze River (the geographical borderline to northern and southern parts of China) or were largely derived from epidemiological surveys without laboratory serum tests. Conceivably, these data do not fully reflect the overall situation in this large country. In this study, we present our investigational findings about sensitization to Bermuda grass, Timothy and *Humulus scandens* in a cohort of southern Chinese patients with allergic rhinitis and/or asthma, and investigate the molecular components causing specific sensitization. We expect that our data about sensitization to the subtropical Bermuda and temperate Timothy grass pollen allergens based on climate and plant distributions in this region would offer important clues to prevention and allergen immunotherapy of grass pollen allergy in China.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Institutional Review Board of First Affiliated Hospital (GYFY-2008-02-23), Guangzhou Medical University. Written informed consent was obtained from all adults and legal guardians of the participating children. The present study has been registered in the Chinese Clinical Trial Registry (<http://www.chictr.org/cn/>, registration number: ChiCTR-DCC-13004003).

2.2. Patients

This was a prospective study conducted between January 2013 and June 2015 using Allergy Information Repository of State Key Laboratory of Respiratory Disease (AIR-SKLRD) in China. The AIR archives thus far the largest dataset of patients who were referred for allergen tests from Greater Guangzhou (city proper and its outskirts) of southern China (Sun et al., 2014; Zeng et al., 2015). During the study period, there were consecutively 282 patients with allergic rhinitis and/or asthma in AIR database, who underwent serum grass allergen specific immunoglobulin E (sIgE) tests at our laboratory. These patients were aged 1–87 years old, with a physician-diagnosis of mild to moderate rhinitis with or without asthma based on Allergic Rhinitis and Its Impact on Asthma (ARIA) (2015) and Global Initiative for Asthma (GINA) (2015) guidelines. Finally, 258 of these subjects were included as the study group, while 24 patients were excluded because of tested grass pollens

being irrelevant to this study ($n = 7$), refusal to participation ($n = 4$), immunodeficiency ($n = 2$), current use of allergen immunotherapy (AIT) or immunomodulatory agents ($n = 9$), or parasitic infections (ascariasis, $n = 2$). As such, the study group comprised 92 cases of asthma, 78 of allergic rhinitis, and 88 of concomitant allergic rhinitis and asthma, or 43 children (< 14 years) and 215 adults (≥ 14 years) (Fig. 1). A contemporary cohort of 88 patients with other allergies such as eczema, allergic dermatitis and food allergies, or 29 children and 59 adults, were finally recruited as the control group, according to the same exclusion criteria. In addition, patients in the control group were free of symptoms of allergic rhinitis or allergic asthma, such as runny nose, nasal congestion, or wheezing. All subjects were confirmed to be AIT-naïve.

2.3. Blood collection, serum processing and storage

Within two days after their first presentation and before any prescriptions were given, all subjects received phlebotomy for a 5 mL venous blood sample which was used for all tests as applicable thereafter. The tested sample was centrifuged for 10 min at 3000 rpm and recovered for the serum supernatant. In order to avoid repeated freezing and thawing, the remaining serum samples were kept in a -80° Celsius refrigerator for long-term storage.

2.4. Detection of grass pollen-specific IgEs

The serum samples were tested for sIgEs to Bermuda grass (g2), Timothy (g6), and Japanese hop (*Humulus scandens*) (w22) using an ImmunoCap1000 system (ThermoFisher Scientific Inc., California, USA). Samples that tested positive for any of these three grass-pollen sIgEs underwent subsequent tests for sensitization to specific allergen component molecules. These included sIgE tests for Bermuda grass antigen Cyn d 1 (g216), Timothy antigens Phl p 1 (g205), Phl p 4 (g208), Phl p 5 (g215), Phl p 6 (g209), Phl p 7 (g210), Phl p 11 (g211), Phl p 12 (g212), and cross-reactive carbohydrate determinants (CCD, o214). Finally, correlation analysis was performed to determine whether there was a relationship in pollen sensitizations between different grasses.

The sIgE concentrations (kU/L) of each allergen were determined. A test was considered positive where the related sIgE concentration equaled to or exceeded 0.35 kU/L. The severity of allergen sensitization was evaluated by five levels (classes 1–5) depending on sIgE levels, as has been described in previous studies (Sun et al., 2014; Zeng et al., 2015).

2.5. Statistical analysis

Data were analyzed with the statistical software package SPSS v13.0. Parametric quantitative data were presented as the mean $\pm$ standard deviation. Non-parametric quantitative data were presented as a median (interquartile range). Categorical data were reported as a percentage showing the proportion of positive results. Proportions were compared between groups with chi-square tests (χ^2). Comparisons between two parametric groups of data were performed using unpaired t -tests. F -tests were used to compare the variance of data amongst the groups. Non-parametric rank sum tests were utilized to compare non-parametric data. Correlation analyses for parametric data were performed using Pearson's tests, with the correlation coefficients expressed as " r ." Correlation analyses between non-parametric data were performed using Spearman's tests, with the correlation coefficients presented as " r_s ." P -values < 0.05 were considered to be statistically significant.

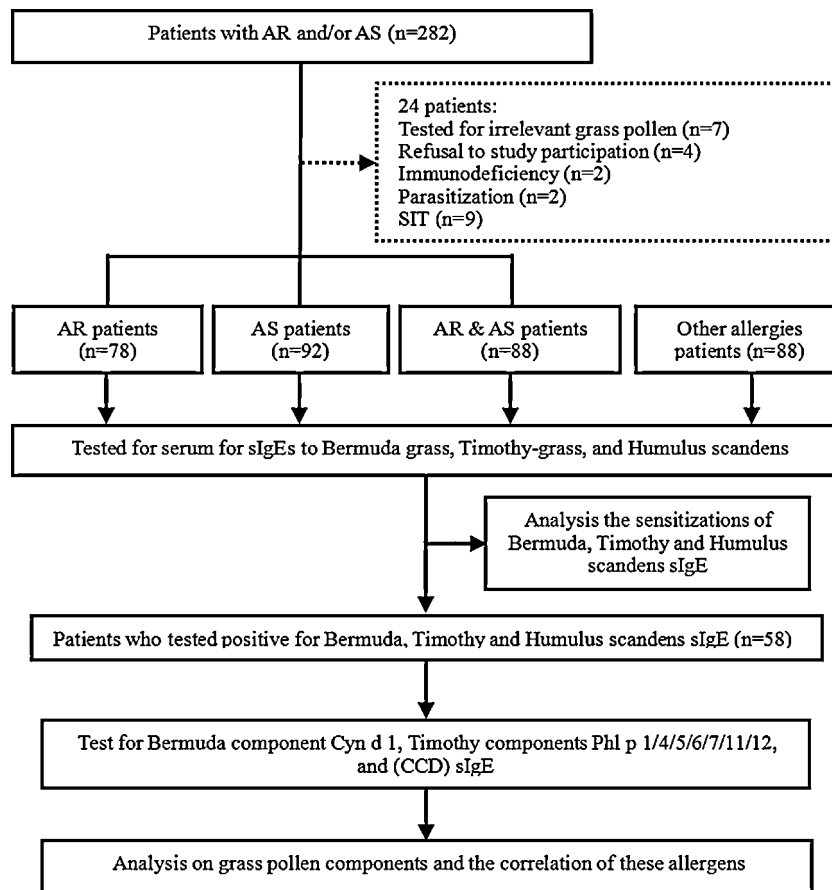


Fig. 1. The flow-chart of the present study.

Table 1

The distribution of patients with positive tests for Bermuda grass, Timothy grass, and *Humulus scandens* sIgE are shown based on disease status and demographic details.

	Rate of positive tests [% (n/pts)]		
	Bermuda grass	Timothy grass	<i>Humulus scandens</i>
Pts with AR and/or AS	22.5% (58/258) †††	13.6% (35/258) †	7.0% (18/258)
Pts with AR	21.8% (17/78) ††	10.2% (8/78)	7.7% (6/78)
Pts with AS	22.8% (21/92) ††	14.1% (13/92) †	5.4% (5/92)
Pts with AR and AS	22.7% (20/88) ††	15.9% (14/88)	8.0% (7/88)
Age			
≤14 years	18.6% (8/43)	14.0% (6/43)	7.0% (3/43)
>14 years	23.3% (50/215)	13.5% (29/215)	7.0% (15/215)
Gender			
Male	25.7% (35/136)	13.9% (19/136)	7.4% (10/136)
Female	18.8% (23/122)	13.1% (16/122)	6.6% (8/122)
Pts with other allergy	4.5% (4/88) **	1.1% (1/88) **	0*

Pts: Patients; AR: Allergic rhinitis; AS: Asthma. A higher proportion of patients with allergic respiratory diseases were positive for Bermuda grass ($\chi^2 = 14.35$, $P < 0.001$), Timothy grass ($\chi^2 = 10.88$, $P < 0.001$) and *Humulus scandens* ($\chi^2 = 5.14$, $P = 0.02$) compared with the other allergy control group, * $P < 0.05$, ** $P < 0.01$. The proportion of patients with a positive Bermuda grass test was significantly higher than the proportion positive to Timothy grass ($P < 0.01$), † $P < 0.05$ and *Humulus scandens* ($P < 0.01$), †† $P < 0.01$. The proportion of patients with a positive Timothy grass test was significantly higher than the proportion positive to *Humulus scandens* ($P < 0.05$), †† $P < 0.05$. The proportion of positive tests to Bermuda grass, Timothy grass, and *Humulus scandens* sIgE did not vary significantly between the minor and adult groups ($\chi^2 = 0.07$, $P = 0.79$; $\chi^2 = 0.06$, $P = 0.80$; $\chi^2 = 0.11$, $P = 0.74$). Rates of grass pollen sensitivities were not significantly different between male and female patients ($\chi^2 = 1.338$, $P = 0.25$; $\chi^2 = 0.063$, $P = 0.80$; $\chi^2 = 0.38$, $P = 0.54$). Similarly, no significant differences in sensitivity rates were observed between the rhinitis and asthma group ($\chi^2 = 0.030$, $P = 0.985$; $\chi^2 = 1.165$, $P = 0.558$; $\chi^2 = 0.528$, $P = 0.768$).

3. Results

3.1. The distribution of patients with positive tests for Bermuda grass, Timothy grass, and *Humulus scandens* sIgE

In the present study, there were 136 males and 122 females in the study group, compared with 45 males and 43 females in the control group. Of patients with allergic rhinitis and/or asthma,

22.5% were Bermuda grass sIgE-positive, 13.6% Timothy sIgE-positive, and 7.0% *Humulus scandens* sIgE-positive. The proportion of patients with allergic rhinitis and/or asthma sensitized to any pollen was significantly higher than that in the control group. All Bermuda grass sIgE-negative patients also tested negative for Timothy grass and *Humulus scandens* sIgE. The sensitizations of patients are shown in Table 1.

Table 2

The demographic details and rates of allergen sensitization in the study group of patients who tested positive for Bermuda grass sIgE (N = 58).

Variables	Patient number (%)
Gender	
Male	35 (60.3)
Female	23 (39.7)
Age (years)	
1–5	4 (6.9)
5–14	4 (6.9)
14–40	7 (12.1)
40–60	32 (55.2)
>60	11 (18.9)
Bermuda sIgE ^a reactivity	
Class 1	20 (34.5)
Class 2	21 (36.2)
Class 3	12 (20.7)
Class 4	3 (5.2)
Class 5	2 (3.4)
Cyn d 1 sIgE ^b positive [n (%)]	31 (53.4)
CCD sIgE ^c positive [n (%)]	24 (41.4)

Of these patients, nearly 74.1% were aged above 40 years old, and over 91.4% presented classes 1–3 sIgE response to Bermuda grass.

^a Bermuda sIgE levels: 1.16, 0.57–4.77 kU/L (median, IQR).

^b Cyn d 1 sIgE levels: 0.47, 0.06–1.83 kU/L (median, IQR).

^c CCD sIgE levels: 0.23, 0.02–2.47 kU/L (median, IQR).

3.2. The demographic details and rates of allergen sensitization for 58 patients who tested positive for Bermuda grass sIgE in the study group

As shown in Table 1, a significantly higher rate of sensitization to Bermuda grass was noted in patients with allergic rhinitis and/or asthma (n = 58, 22.5%) than in those with other allergies (n = 4, 4.5%). The demographic details and rates of pollen sensitization were assessed amongst the 58 patients who tested positive for Bermuda grass sIgE in the study group. Of these patients, nearly 75.0% were aged above 40 years old, and over 90% presented classes 1–3 sIgE response to Bermuda grass (Table 2). Thirty-five of these 58 patients were sIgE-positive to Timothy grass, and 18 to *Humulus scandens*. A positive test for Cyn d 1 was observed in 31 patients, and 24 were positive for CCD. In addition, the sIgE response levels of Bermuda grass were significantly higher than that for Timothy grass or *Humulus scandens* (Fig. 2).

3.3. The relationship between Bermuda-sIgE levels and sensitization to other grass allergens and components

Patients who tested sIgE-positive to Bermuda grass were further assessed for co-sensitization to other grass allergens and components after stratified according to sIgE response (classes 1–5). Among the patients with Bermuda grass class-1 sIgE reactivity, the likelihood of positive tests for Cyn d 1, Timothy grass, *Humulus scandens*, or CCD was below 30%. In contrast, patients with class-4 Bermuda grass sIgE reactivity had a 67% to 100% chance of concomitant positivity to Cyn d 1, Timothy grass, *Humulus scandens*, or CCD. All patients (100%) with class-5 Bermuda grass sIgE reactivity tested positive for Cyn d 1, Timothy grass, *Humulus scandens*, and CCD (Fig. 3). Bermuda grass sIgE reactivity levels demonstrated a significant correlation with chances of positivity to other grass pollen sIgEs ($r_s = 0.669$, $P < 0.001$). Bermuda grass sIgE levels were significantly higher in Cyn d 1 sIgE positive serum compared with Cyn d 1 sIgE negative patients ($z = 1.711$, $P = 0.006$, 2.25 vs. 0.67 kU/L). Bermuda grass sIgE levels were also significantly higher in Timothy grass sIgE positive patients ($z = 2.519$, $P < 0.001$, 3.76 vs. 0.60 kU/L) and *Humulus scandens* sIgE positive patients ($z = 1.678$, $P = 0.007$,

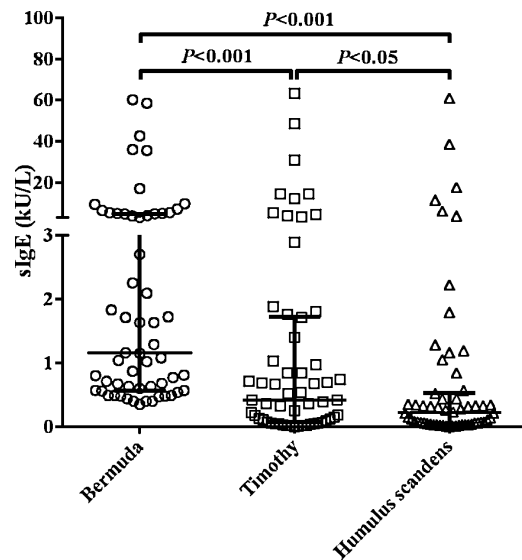


Fig. 2. Specific IgE levels to different grass pollens are shown by the median (IQR). Bermuda grass sIgE levels (1.16, 0.57–4.77 kU/L) were significantly higher than Timothy grass sIgE (0.42, 0.08–1.72 kU/L) and *Humulus scandens* sIgE (0.23, 0.04–1.21 kU/L) ($z = 2.85$, $P < 0.001$). Timothy grass sIgE levels were significantly higher than *Humulus scandens* sIgE levels ($z = 1.486$, $P = 0.024$).

4.80 vs. 0.79 kU/L), compared with Timothy and *Humulus scandens* sIgE negative patients.

3.4. The sensitization pattern of timothy components

Timothy grass sensitization was further assessed by total Timothy grass sIgE and sIgE to Timothy grass allergen components. Of the 35 Timothy grass sIgE positive patients, the most frequently detected Timothy grass allergen component was Phl p 4 (100%, 35/35), followed by Phl p 1 (17.1%, 6/35). Three patients each (3/35, 8.6%) were positive for any one of Phl p 5, 6, 7, 11, and 12 (Table 3). Timothy grass extract sIgE level and Phl p 4 sIgE level were significant higher than Phl p 1, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p 12 sIgE level ($P < 0.05$). There was no significant difference in sIgE levels between Timothy grass extract and Phl p 4 ($P > 0.05$) (Fig. 4). Serum Phl p 4 sIgE levels correlated strongly with Timothy grass and CCD sIgE levels ($r_s = 0.941$ and 0.928 , $P < 0.001$) (Table 4).

3.5. The relationship between CCD and pollen allergen sensitization

The relationship between CCD and pollen allergen sensitization was assessed. 41.4% (24/58) of Bermuda grass positive patients and 68.6% (24/35) of timothy sensitized patients were sIgE positive to CCD. Spearman's rank correlation analysis showed that a significant association in sensitization between CCD and other grass allergens. The Bermuda grass, Timothy grass, and *Humulus scandens* sIgE levels well correlated among one another (Table 4).

4. Discussion

Bermuda grass is a common grass species in tropical and subtropical regions. In Europe, while Timothy grass is mainly found in the United Kingdom and northern parts of the continent, Bermuda grass grows only in southern Europe (D'Amato et al., 2007). In China, Bermuda grass is widely distributed south to the Yangtze River, especially in several China's southern provinces, such as Guangdong, Guangxi, and Taiwan (Jianxiu et al., 2003; Huixia and Jiabin 2005). A previous study indicated that ~27% of asthma patients in Taiwan were sensitive to Bermuda grass pollen allergens (Kao et al.,

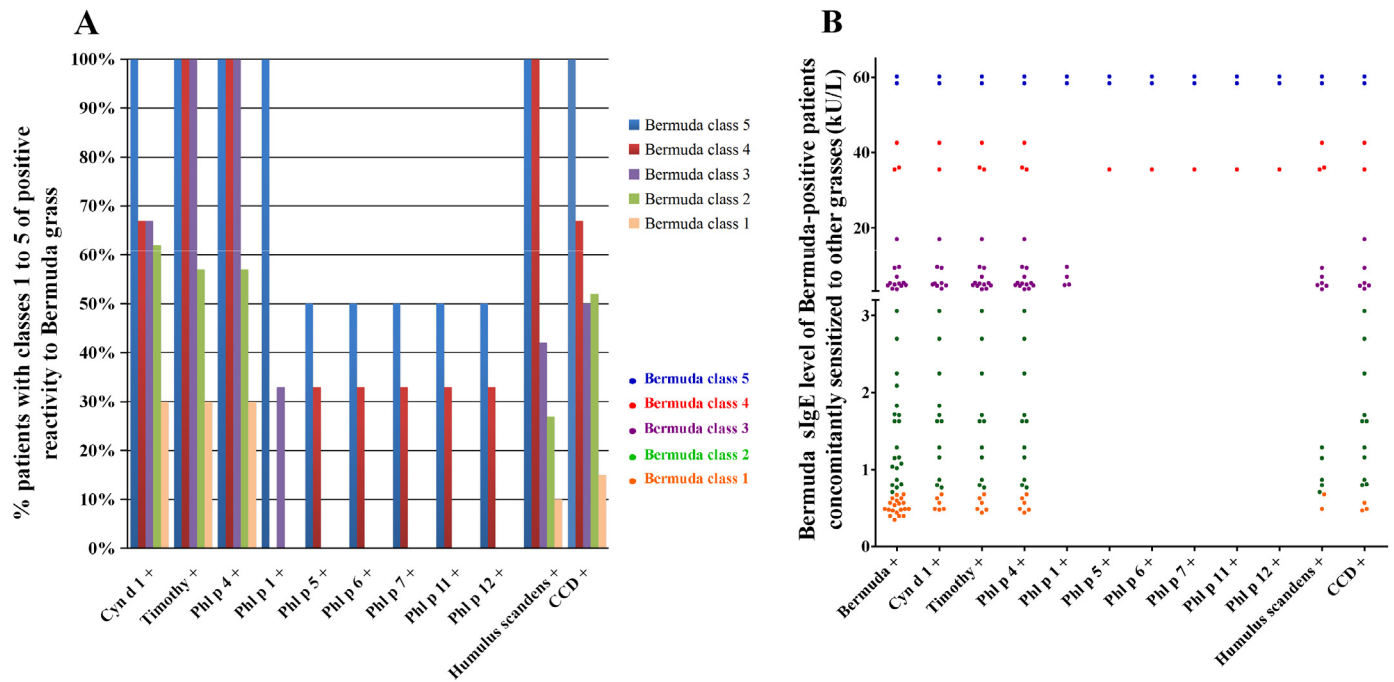


Fig. 3. Co-sensitization to Timothy, *Humulus scandens*, CCD and allergen components among patients positive to Bermuda grass sIgE (N = 58).

Panel A: showing the percentage of patients with classes 1–5 of positive reactivity to Bermuda grass (%) and concomitant sensitization to other grass allergens or components; Panel B: showing absolute Bermuda grass sIgE concentrations of Bermuda-positive patients (in colored dots according to sIgE reactivity classes) with concomitant sensitization to other grass allergens or components;

Sensitization to Timothy and *Humulus scandens* was observed only in patients sensitized to Bermuda grass. Among the patients with Bermuda grass class-1 sIgE reactivity, the likelihood of positive tests for Cyn d 1, Timothy grass, *Humulus scandens*, or CCD was below 30%. In contrast, patients with class-4 Bermuda grass sIgE reactivity had a 67% to 100% chance of concomitant positivity to Cyn d 1, Timothy grass, *Humulus scandens*, or CCD. All patients (100%) with class-5 Bermuda grass sIgE reactivity tested positive for Cyn d 1, Timothy grass, *Humulus scandens*, and CCD.

Table 3

Timothy grass sensitization according to sIgE positivity and levels of Timothy grass components (N = 35).

Patients (%)	Phl p 4	Phl p 1	Phl p 5	Phl p 6	Phl p 11	Phl p 7	Phl p 12
28 (80.0%)	+						
4 (11.4%)	+	+					
1 (2.9%)	+		+	+	+	+	+
2 (5.7%)	+	+	+	+	+	+	+
Total	35 (100%)	6 (17.1%)	3 (8.6%)	3 (8.6%)	3 (8.6%)	3 (8.6%)	3 (8.6%)
sIgE ^a (kU/L)	0.52, 0.06–2.02	0.02, 0.01–0.10	0.04, 0.03–0.24	0.12, 0.03–0.34	0.05, 0.02–0.27	0.03, 0.02–0.36	0.10, 0.04–0.28

+: Positive tests; ^a, sIgE levels (kU/L) are shown as median, IQR. Of the 35 Timothy grass sIgE positive patients, 80.0% were positive to Phl p 4 only, while 11.4% were positive to Phl p 4 and Phl p 1. Moreover, 2.9% of patients were sensitized to Phl p 4, Phl p 5, Phl p 6, Phl p 11, Phl p 7, and Phl p 12. 5.7% of patients were sensitized to all antigens (Phl p 4, Phl p 1, Phl p 5, Phl p 6, Phl p 11, Phl p 7, and Phl p 12).

2005). Despite the wide distribution of Bermuda grass in southern China, there have been few studies published to date assessing grass pollen sensitization in this vast part of Asian continent.

Geographically, Guangzhou is the capital metropolis and located closely to the center of China's Guangdong Province that faces the South China Sea. In Guangzhou, the perennial climate is typically humid and subtropical, with plentiful rains and high temperatures during the summers. Such a natural environment is flourished by rampant growth of numerous trees and grass forming a complexity of flora seldom studied for local allergology. In Greater Guangzhou where our study cohort was living, Bermuda grass has been a common grass species widely grown for multiple purposes (sports turfs, park lawns and golf courses) along with the prospering economy that allowed for improvement of urban infrastructure over the past decades. Comparatively, Timothy grass and *Humulus scandens* are not common, even non-existing in Guangzhou. Sporadic data from botanists located minimal growth of Timothy in northern mountainous areas of Guangdong Province (220 miles to the northwest of Guangzhou) used for cattle breeding. Despite a common source of grass allergen in northern China and a harmful weed that ham-

pers agricultural production, agronomist reports about *Humulus scandens* in Greater Guangzhou are so far lacking.

The results of this study showed a considerably high prevalence of sensitization to Bermuda grass in a cohort of patients with allergic rhinitis and/or asthma in Guangzhou, the largest city of southern continental China. The rate of Bermuda grass sensitization in this group was 22.5%, which is slightly lower than 27% in Taiwan (Kao et al., 2005). Timothy grass was the next most frequent pollen allergen, followed by *Humulus scandens*. Sensitization to these two grass species was observed only in patients sensitized to Bermuda grass. Moreover, we found that the sIgE levels for Bermuda grass in sensitized subjects were significantly higher than those for Timothy grass and *Humulus scandens*, and subjects with higher Bermuda grass sIgE levels were more likely to be sensitized to Timothy grass and *Humulus scandens*. These findings echoed a study from Australia where Bermuda grass IgE concentrations were noted to be higher than temperate grass pollen specific IgE (Nony et al., 2015). In South Korea, a study showed that the rates of Bermuda grass sensitization were significantly higher in those sensitized to *Humulus scandens* pollen compared to those who were not (Park et al., 2001).

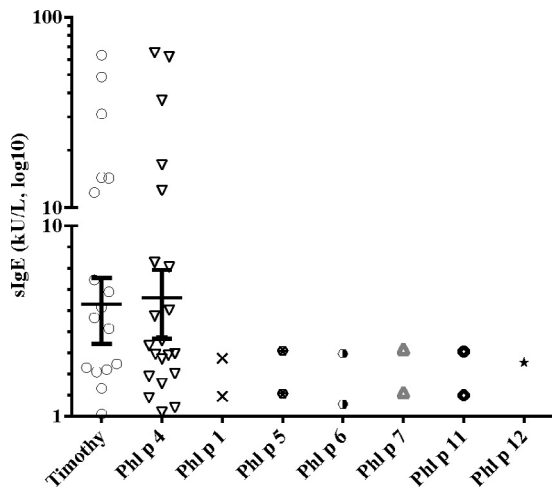


Fig. 4. IgE levels of Timothy components among patients to Timothy grass extract (N = 35). Timothy grass IgE levels (kU/L) are shown as log₁₀. Timothy grass extract IgE level and Phl p 4 IgE level were significant higher than Phl p 1, Phl p 5, Phl p 6, Phl p 7, Phl p 11 and Phl p 12 IgE level ($P < 0.05$). There was no significant difference in IgE levels between Timothy grass extract and Phl p 4 ($P > 0.05$).

Table 4

Correlation coefficients^a between cross-reactive carbohydrate determinants and grass pollen allergens.

Allergen	Bermuda	Timothy	<i>Humulus scandens</i>	CCD
Bermuda	–	0.709**	0.682**	0.565**
Cyn d 1	0.583**	0.787**	0.751**	0.865**
Timothy	0.709**	–	0.921**	0.856**
Phl p 4	0.645**	0.941**	0.886**	0.928**
Phl p 1	0.616**	0.681**	0.712**	0.507**
Phl p 5	0.460	0.273	0.590*	0.624*
Phl p 6	0.445	0.306	0.583*	0.569
Phl p 7	0.681*	0.481	0.632*	0.531
Phl p 11	0.589*	0.372	0.620*	0.568
Phl p 12	0.671*	0.411	0.565	0.446
<i>Humulus scandens</i>	0.682**	0.921**	–	0.853**

^a Presented as correlation coefficient r_s ; Correlation analyses between parametric datasets were performed using Spearman's tests.

** $P < 0.01$.

* $P < 0.05$.

One Australian study assessing cross-inhibition tests of subtropical grass pollen allergen showed that Bermuda grass IgE could not be inhibited by other grass pollens (including Timothy grass); on the contrary, Bermuda grass could suppress Timothy grass IgE, indicating that Bermuda grass may have an independent epitope, and can induce nonspecific cross-reactivity in serum containing Timothy grass IgE (Davies et al., 2012).

Cyn d 1 belongs to grass pollen group 1 antigens that were determined to be acidic glycoproteins with a molecular weight of 31–35 kDa. It has a ~64% homology in amino acid sequencing compared with Phl p 1 antigens (Duffort et al., 2008), and is considered as one of the most prominent Bermuda grass antigens. The 32-kDa Cyn d 1 has also been frequently used as a biomarker of sensitization to Bermuda grass. It is estimated that 76–100% of Bermuda grass allergic patients are Cyn d 1 positive (Frenguelli et al., 2010; Shen et al., 1988), and this is also reflective of clinical symptoms of allergy (Smith et al., 1996). In this study, 53.4% of Bermuda grass IgE positive patients were also positive to Cyn d 1. This lower rate of Cyn d 1 positivity might be due to the fact that Bermuda grass IgE levels were mostly classes 1–3 among these patients in our study. Higher Bermuda grass IgE levels correspond with higher rates of positive tests for Cyn d 1, as we have also noted. This indicated that patients who have a high level of Bermuda grass IgE may be

more likely to be sensitized to the genuine allergen component of Bermuda grass (Cyn d 1).

This study showed that 60.3% of Bermuda grass IgE positive patients were also positive for Timothy grass IgE. All Timothy grass IgE positive patients were also positive to the specific Timothy grass antigen, Phl p 4. However, only 17.1% of patients were positive to the antigen Phl p 1, which was obviously different from data in Europe where the rate of positivity to Phl p 1 is much higher compared with Phl p 4. Previously, it was shown that there are substantial variations in the two major Poideae pollen allergens Phl p 1 and Phl p 5, even amongst five phylogenetically related temperate grass species (Chabre et al., 2010). In that French study, timothy grass pollen antigens Phl p 1 and Phl p 5 were the most predominant grass pollen allergens (Chabre et al., 2010). Investigators from Zimbabwe in Central Africa looked at local prevalence of positivity to Timothy components and compared their findings with those from Austria (Westritschnig et al., 2003). According to that study, the IgE recognition frequency of Phl p 1 (80%) was comparable with 96% in Austria, but those for Phl p 5 (27%) and Phl p 6 (15%) were much lower than the data from Austria (100% and 52%, respectively). Apparently, there seemed to be similarities and differences in data among the temperate Austria, the tropical Zimbabwe and southern China's subtropical Guangdong, which may well emphasize the regional specificity in allergy to grass pollens.

Two studies in Italy have showed that Phl p 1 sensitization is present in 90% to 92% of patients allergic to Timothy grass (Rossi et al., 2001; Mari 2003). The rate of Phl p 4 sensitivity varies in terms of geographical location, ranging from 54% to 92% (Suck et al., 2000; Fischer et al., 1996; Stumvoll et al., 2002; Tripodi et al., 2012). Phl p 4 has a molecular weight of 54 kDa. It is considered to be highly cross-reactive with other grass allergens, and may cause only weak immune response in the majority of allergic patients (Tripodi et al., 2012). Some Phl p 4 positive patients show direct reactivity to CCD, which may be associated with cross-reactivity (Andersson and Lidholm 2003; Zafred et al., 2013). The relationship of this reactivity with clinical symptoms is uncertain. In Austria, it has been shown that about 20% of patients with allergy are CCD positive, and this is true for allergy to nearly all plants and invertebrates (Atmann, 2007). CCD can cause extensive cross-reactivity between different allergen sources. It can be a potential source of interference and may cause nonspecific false-positivity with *in vitro* tests (Zafred et al., 2013; Atmann 2007). Among these allergens, the molecules from *Phleum pratense*, Phl p 1, Phl p 2, Phl p 5, and Phl p 6, and their homologs in other grasses, are known as major allergens (Tripodi et al., 2012). They are also the selective markers used for Timothy grass specific immunotherapy (SIT). Similarly, Cyn d 1 is used as a selective marker for Bermuda grass SIT (Popescu, 2014). In our study, the rate of positive testing for Phl p 1/5/6/7/11/12 was quite low. However, of Timothy-positive patients, all were positive for Phl p 4 (100%), including 73.4% who were positive to Phl p 4 alone. A European study of 176 different Timothy grass antigens showed that the proportion of Phl p 1 positive patients who were allergic to Timothy grass was 90%, and 20.8% of patients were Phl p 1 positive alone (Tripodi et al., 2012). However, it was observed in our cohort from Guangdong that the rate of Phl p 1 positivity was only 17.1%, and none patient was positive to Phl p 1 alone. This result was in surprising contrast to findings from previous European studies. The clinical relevance by this observation could be important, because the patients in our study group were not to be advised to undergo Timothy grass SIT therapy (Stumvoll et al., 2002), highlighting the need to use allergen component-resolved diagnosis for allergy prevention and treatment.

Noticeably, as we have presented in this study that positivity to Timothy and some to Bermuda grass were due to CCD cross-reactivity, it would be an interesting question about the original sensitizing allergen, or if there are other plant sources of CCD

to which these patients may be sensitized. In tropical Philippine, a study using microarray techniques revealed that natural Phl p 4 and Cyn d 1 molecules contain N-linked carbohydrates which are present as CCD in a number of allergen sources like insects, plants, and animals (Cabauatan CR et al., 2014). Those investigators assumed that the tropical flora (grasses in particular) might generate highly glycosylated allergens where protein epitopes are embedded by the carbohydrates, and primarily lead to IgE sensitizations against carbohydrate epitopes. We speculated this might partly be of an answer. Of the Phl p 4 positive patients in our study, 65.7% were also CCD positive, and Phl p 4 had a good correlation with CCD. This indicated that CCD might cause cross-reactivity between grass pollen allergens (Andersson and Lidholm, 2003), and may also explain why Timothy grass sIgE was detected in this cohort of Guangzhou patients. However, *in vitro* cross-inhibition assays are worthwhile to confirm this hypothesis. Further insight to this question would inspire future collaborations among allergists, botanists and agronomists, given the inadequately explored complexity of subtropical flora in this geographical region of southern China.

Several limitations of this study should be acknowledged. Firstly, it seems that testing this cohort for sIgE reactivity with relevant recombinant subtropical grass pollen allergen components that lack CCD, could be more informative about Bermuda grass and Cyn d 1 sensitization in these patients. Secondly, our study lacked cross-inhibition assays, which would otherwise offer more data about the degree of CCD involvement in the sIgE reactivity with Bermuda grass pollen and/or Cyn d 1 and Phl p 4. Unfortunately, owing to the late introduction of component-resolved diagnosis of allergens in China, several commercial products of subtropical grass pollen components in the southern region are unavailable or have not been registered for research purpose until recently. Nevertheless, to the latest of our knowledge, this study may be the first using component-resolved diagnosis on sensitization to grass pollen allergens in southern China. Future studies are highly expected to continue this topic with efforts that circumvent the limitations in our study.

5. Conclusions

In southern China, patients with allergic rhinitis and/or asthma are frequently sensitized to grass pollen allergens. The rate of Bermuda grass sIgE positivity was 22.5%, Timothy grass sIgE was positive in 13.6% of patients, and *Humulus scandens* was positive in 7.0%. Higher Bermuda grass sIgE levels were more likely to be associated with sensitivity to Timothy grass and *Humulus scandens* pollens. Good correlations were demonstrated between CCD and grass pollen allergens. This indicates that CCD may cause cross-reactivity between grass pollen allergens. However, *in vitro* inhibition assays are required to confirm the relationship between CCD and grass pollen allergy.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceived and designed the experiments: SBQ, ZGQ. Performed the experiments: LWT, WNL, ZPY, LJY. Analyzed the data: LWT, HHM, ZGQ. Reviewed the data: SBQ. Wrote the paper: LWT, HHM, ZGQ.

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