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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.

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 pretense*), 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 subtropical 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 (GYFYY-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 ± 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.