

A comprehensive analysis of the components of common weed pollen and related allergens in patients with allergic diseases in southern China

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ABSTRACT

Background: Pollen allergens are vital contributors to allergic diseases. The frequency and coreactivity pattern of allergens are closely related to geographical distribution.

Objective: In this study, we aimed to characterize the prevalence of the molecular components of the common weed pollen allergens, birch pollen, walnut, and cross-reactive carbohydrate determinant (CCD), as well as investigate the relationship between the allergens and CCD in Chinese pollen-sensitized patients with allergic diseases.

Methods: Based on previous vegetation surveys, serum samples from 165 pollen-sensitized patients with allergic diseases in Guangdong Province in southern China were used to test 19 crude allergen extracts, their components, and CCD using component-resolved diagnosis (CRD). Moreover, the potential associations among CCD, allergens, and their components were described.

Results: In the 165 samples, the most common sensitized allergens were goosefoot (43.0%), ragweed (40.6%), walnut (37.6%), walnut tree (37.6%), and mugwort (37.0%), followed by platane (35.2%), cocklebur (27.9%), and birch (24.2%). The positivity rate of CCD was 39.4%. Among the samples positive for mugwort, 11 (18.0%), 15 (24.6%), and 15 (24.6%) were positive for Art v 1, Art v 2, and Art v 3, respectively. Among the 67 patients sensitized to ragweed, only five (7.5%) were positive for Amb a 1. In the 40 patients sensitized to birch, Bet v 2 had the highest positivity rate (40.0%). There were 62 patients who were sensitized to walnut. Their components had a lower positivity rate (less than 15%). The hierarchical clustering and optimal scale analysis showed that Art v 4 and Bet v 2 were closely related, and 91.9% of CCD-positive samples were polysensitized. Meanwhile, Spearman's rank correlation method showed that CCD was closely correlated with the sensitization of crude allergen extracts, and there was a low correlation between CCD and allergen components. CCD was highly correlated with goosefoot, ragweed, and walnut trees ($r > 0.8$). Moreover, there was a strong relationship between the levels of Jug r 3 and Art v 3 ($r = 0.78$; $P < 0.001$).

Conclusions: In southern China, the weed pollens (ragweed, cocklebur, and goosefoot) exhibited higher positivity rates in adults and had a stronger relationship with CCD but not with mugwort. The positivity rate of allergen components was not high. CCD-positive samples were always polysensitized.

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1. Introduction

Allergic diseases affect more than a quarter of the population in developed countries. Their prevalence in developing countries has also been rapidly increasing over the last decades. Weed pollen is an important type of seasonal allergen and a potential health hazard. Among pollen-producing weeds, mugwort and ragweed are common allergenic weeds belonging to the Asteraceae or Compositae plant family. The goosefoot of the Chenopodium family and the cocklebur of the Xanthium family are also associated with allergic diseases (Weber, 2015; Weber, Cocklebur 2004). According to previous vegetation investigations, mugwort pollen is the most allergenic pollen in the northern region of the Yangtze River in China (Tang et al., 2015; Wang et al., 2021). Recent research indicates that component-resolved diagnosis (CRD) helps evaluate the potential risk of mugwort-associated allergic asthma in Chinese patients with a mugwort pollen allergy in northern China (Gao et al., 2019). Ragweed is particularly harmful to public health because each plant produces a large amount of pollen (≤ 1 billion grains a year), and its allergenic potential is high. Ragweed is one of the common pollen allergens in northern China (Wang et al., 2021). However, studies on their components are scarce in southern China. Patients allergic to weed pollens are frequently polysensitized to diverse plant sources. Therefore, molecule-based approaches are especially valuable for a precise diagnosis.

Previous studies have found that patients sensitized to birch pollen are also cosensitized to weed pollen, while another study showed that some people sensitized to mugwort are also cosensitized to walnut (Skypala et al., 2019). Cosensitization to these pollens could result from similar or cross-reactive proteins present in the pollens. However, tests based solely on allergen extracts do not always discover the source of IgE sensitization, especially when cross-reactive allergens are involved and patients may be sensitized to multiple sources of homologous allergens. (Pomés et al., 2015). Nearly thirty years ago, molecular cloning and analysis of the molecular structure of allergens emerged and improved the method of *in vitro* diagnosis with the use of panels of purified individual allergens instead of extracts. CRD, used within the context of the patient's clinical history, is an effective method to identify the patient's IgE sensitization profile.

In this study, we aimed to characterize the prevalence of the components of common weed pollens and analyse the relationship between birch pollen allergen, walnut allergen, and cross-reactive carbohydrate determinant (CCD) in southern China by using CRD to guide clinical diagnosis.

2. Materials and methods

2.1. Serum sample collection

Serum samples and patient demographics were obtained from the National Clinical Research Center of Respiratory Disease located at the First Affiliated Hospital of Guangzhou Medical University.

Samples were selected from patients who had a history of contact with common allergens in China and exhibited reactivity to at least two pollen allergens using the ImmunoCAP assay (ThermoFisher, Waltham, MA). Samples from patients who had received allergen immunotherapy or had a history of parasitic infection, autoimmune disease, obstructive pulmonary disease, or lung cancer were excluded from the study.

2.2. Measurement of allergen-sIgE

Serum sIgE of mugwort (*Artemisia vulgaris*), Art v 1, Art v 3, Art v 4, ragweed (*Ambrosia*), Amb a 1, birch (*Betula*), Bet v 1, Bet v 2, Bet v 4, walnut, Jug r 1, Jug r 2, Jug r 3, Jug r 4, cocklebur (*Xanthium strumarium*), goosefoot (*Chenopodium album*), walnut tree (*Juglans regia*), platane (*Platanus sp*), and CCD was measured using the EUROIMMUN system following standard protocols. The quantification threshold was

0.35 kUA/L for sIgE. The sIgE reactivity was categorized quantitatively into six classes (classes 1–6) depending on the sIgE concentration as previously described (Luo et al., 2016).

2.3. Data analysis

Statistical analyses and the generation of figures were performed using Excel 2016 and SPSS 26.0. Normally distributed variables, such as age, were summarized with the mean $\pm$ standard error. Qualitative information, such as the positivity rate, was summarized in terms of a percentage or frequency. The intergroup difference was calculated using the chi-square test (χ^2). Hierarchical clustering analysis was adopted to classify all variables by analysing the similarity or dissimilarity of data. Optimal scale analysis was used to verify the correlation by changing the original variable. Spearman's rank correlation was used as a nonparametric test of correlation, and the correlation coefficient was denoted as "r". $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. The demographic details and positive rates of allergen extracts

A total of 165 patients, aged 29.56 ± 22.94 years, were enrolled in this study. Among them, 96 were adults (≥ 18 years), and 69 were children (< 18 years). The most common sensitized allergens were goosefoot (43.0%, 71/165), followed by ragweed (40.6%, 67/165), walnut (37.6%, 62/165), walnut tree (37.6%, 62/165), mugwort (37.0%, 61/165), platane (35.2%, 58/165), cocklebur (27.9%, 46/165), and birch (24.2%, 40/165). The positive rate of CCD was 39.4% (65/165). The sIgE-positive rates of ragweed (49.0% vs. 20%, $\chi^2 = 6.640$, $P = 0.010$), cocklebur (34.4% vs. 18.8%, $\chi^2 = 4.818$, $P = 0.028$), goosefoot (53.1% vs. 29.0%, $\chi^2 = 9.543$, $P = 0.002$), and CCD (47.9% vs. 27.5%, $\chi^2 = 6.984$, $P = 0.002$) were higher in adults than in children (Table 1).

3.2. The distribution of the positive allergen extracts and their components

Among those who were sIgE-positive to mugwort, 11 (18.0%), 15 (24.6%), and 15 (24.6%) were sIgE-positive to Art v 1, Art v 2, and Art v 3, respectively. Bet v 2 had the highest positivity rate (40.0%) in those patients who were sIgE-positive for birch, followed by Bet v 1 (17.5%) and Bet v 4 (12.5%). Only three (7.5%) of them were positive for Bet v 1,

Table 1
Baseline characteristics of 165 patients from southern China.

Characteristic	Children	Adult	P
Total (N)	69	96	–
Sex (male/female)	53/16	58/38	0.027*
Age (mean $\pm$ S.D.)	7.21 $\pm$ 4.26	47.24 $\pm$ 15.53	–
Disease n (%)			
Rhinitis	23(33.0)	30(31.3)	0.062
Asthma	30(43.5)	57(59.3)	
Rhinitis and asthma	7(10.1)	5(5.2)	
Atopic dermatitis	9(13.0)	4 (4.1)	
Positive for allergen n (%)			
mugwort	20(29.0)	41(42.7)	0.072
birch	17(24.6)	23(24.0)	0.920
ragweed	20(29.0)	47(49.0)	0.010*
cocklebur	13(18.8)	33(34.4)	0.028*
goosefoot	20(29.0)	51(53.1)	0.002**
walnut	25(36.2)	37(38.5)	0.763
walnut tree	22(31.9)	40(41.7)	0.201
platane	21(30.4)	37(38.5)	0.282
CCD	19(27.5)	46(47.9)	0.008**

n test number.

CCD: cross-reactive carbohydrate determinant.

* Chi-square tests, ** $P < 0.01$. * $P < 0.05$.

Bet v 2, and Bet v 4 at the same time (Figs. 1 and 2b). For the 67 patients sensitized to ragweed, only five (7.5%) were positive for Amb a 1 (Figs. 1 and 2c). The positivity rates of Jug r 1 (1.6%), Jug r 2 (3.2%), Jug r 3 (12.9%), and Jug r 4 (6.5%) were low in the patients who were sIgE-positive for walnut (Fig. 1).

3.3. The sensitization pattern in patients with pollen-sensitized allergic diseases

Hierarchical clustering analysis divided CCD, allergens, and their components into seven different sensitization clusters (Fig. 3a). The first cluster contained eight elements (ragweed, Amb a 1, cocklebur, goosefoot, walnut tree, platane, and CCD), the second cluster included two elements (birch, and Bet v 1), the third cluster included two elements (Art v 4, Bet v 2), the fourth cluster included four elements (mugwort, Art v 1, Art v 3, and Jug r 3), and the fifth cluster included two elements (Bet v 4, and Jug r 2). Additionally, Jug r 4 was independent of the sixth cluster, and Jug r 1 was independent of the seventh cluster (Fig. 3a).

The optimal scale analysis verified the abovementioned grouping and showed that it was significantly associated with the components of ragweed, goosefoot, cocklebur, walnut, walnut tree, Amb a 1, and platane (Fig. 3b, red circle). Art v 4 and Bet v 2 had a strong correction (Fig. 3b, blue circle).

3.4. Further analysis of cosensitization after hierarchical clustering analysis

The cosensitization of each cluster of elements was analysed using an UpSet plot and a Venn diagram (Fig. 4). Fig. 4a shows that 32.6% (31/95) of patients in the first cluster were positive for six elements (ragweed, cocklebur, goosefoot, walnut tree, platane, and CCD). A total of 91.9% (57/62) of the CCD-positive patients were polysensitized (Fig. 4a). A total of 14.6% (6/40) of patients in the second cluster were sensitized to both birch and Bet v 1 (Fig. 4b). A total of 16.7% (1/6) of patients in the fifth cluster (16.7%) were sensitive to both Bet v 4 and Jug r 2 (Fig. 4c). A total of 3.0% (2/67) of patients in the fourth cluster were positive for mugwort, Art v 1, Art v 3, and Jug r 3, 4.5% (3/67) were sensitized to three components, and 20.9% (14/67) were sensitized to two components (Fig. 4d). Moreover, 93.8% (15/16) of patients in the third cluster were positive for Art v 4 and Bet v 2 (Fig. 4e).

3.5. The relationship between CCD and allergen sensitization

Spearman's rank correlation analysis showed that the sIgE of CCD had significant correlations with the sIgE to goosefoot ($r = 0.88$, $P < 0.001$), walnut tree ($r = 0.81$, $P < 0.001$), cocklebur ($r = 0.76$, $P < 0.001$), walnut ($r = 0.57$, $P < 0.001$), and birch ($r = 0.52$, $P < 0.001$) (Fig. 5). The levels of mugwort-sIgE were strongly correlated with the sIgE of Art v 1 ($r = 0.71$, $P < 0.001$) and moderately correlated with the sIgE of Art v 3 ($r = 0.57$, $P < 0.001$), ragweed ($r = 0.46$, $P < 0.001$), Amb a 1 ($r = 0.47$, $P < 0.001$), cocklebur ($r = 0.46$, $P < 0.001$), and Jug r 3 ($r = 0.40$, $P < 0.001$). Meanwhile, the levels of Jug r 3-sIgE were strongly correlated with the Art v 3-sIgE ($r = 0.78$, $P < 0.001$) (Fig. 5).

4. Discussion

In this study, we investigated the allergens and molecular components of pollen in southern China. It was found that the sensitization patterns of pollen allergens in southern China had significant characteristics, and the pollen allergens were divided into 7 clusters. This study may provide a reference for the clinical selection of allergen detection methods. There were significant correlations among different allergens, which suggests that there may be cross-reactions between pollen allergens. Southern China is in a subtropical climate region that is generally warm, humid, and rainy, and the pollen concentrations is low. Previous studies (Liao et al., 2020) have reported that the positive frequency of mugwort sensitization in southern China is significantly lower than that in northern China (Panzner et al., 2014; Grewling et al., 2018). The positivity rates of Art v 1 (18.0%), Art v 3 (24.6%), and Art v 4 (24.6%) in mugwort-sensitized patients from southern China were significantly lower than those from northern China and many parts of Europe (Pablos et al., 2016); this finding might be related to the geographical differences in the distribution and pollen load of mugwort in China. The frequency of Art v 3 positivity in this study was 24.6%; Gao et al. obtained a similar result in Yunnan in southwest China (Gao et al., 2019). Thus, the frequency of Art v 1 and Art v 3 positivity was similar within the same latitude range, and differed within the same longitude range. Allergic sensitization to ragweed has steadily increased with global warming (Singer et al., 2005; Stinson and Bazzaz, 2006). The positivity rates of ragweed are up to 40.6% in pollen-allergic individuals, which is similar to the finding of Boulet et al. (Boulet et al., 1997). Ninety-five percent of ragweed pollen allergic patients are sensitized to Amb a 1 (King et al., 1981), which represents the major allergen from ragweed

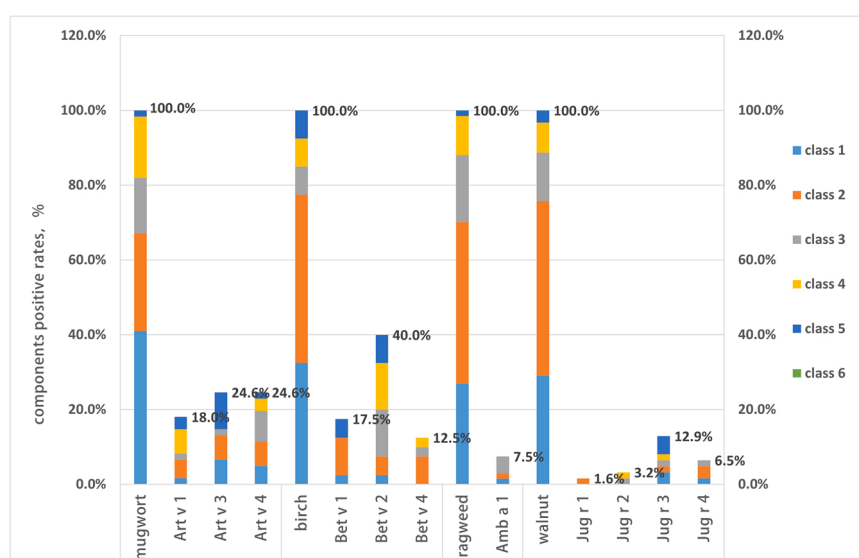
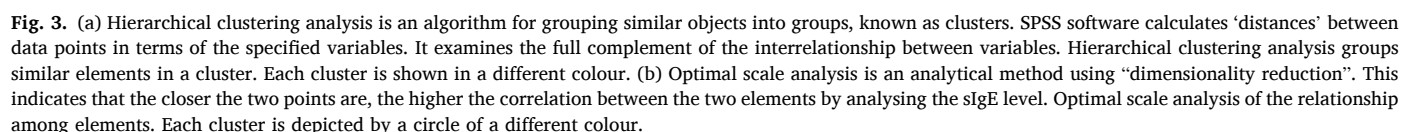
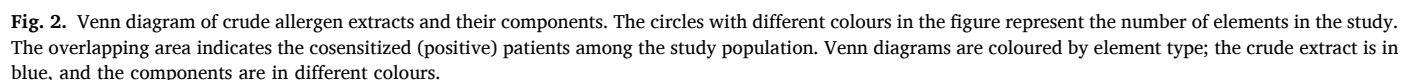


Fig. 1. The positivity rate of crude allergen extracts and their components.



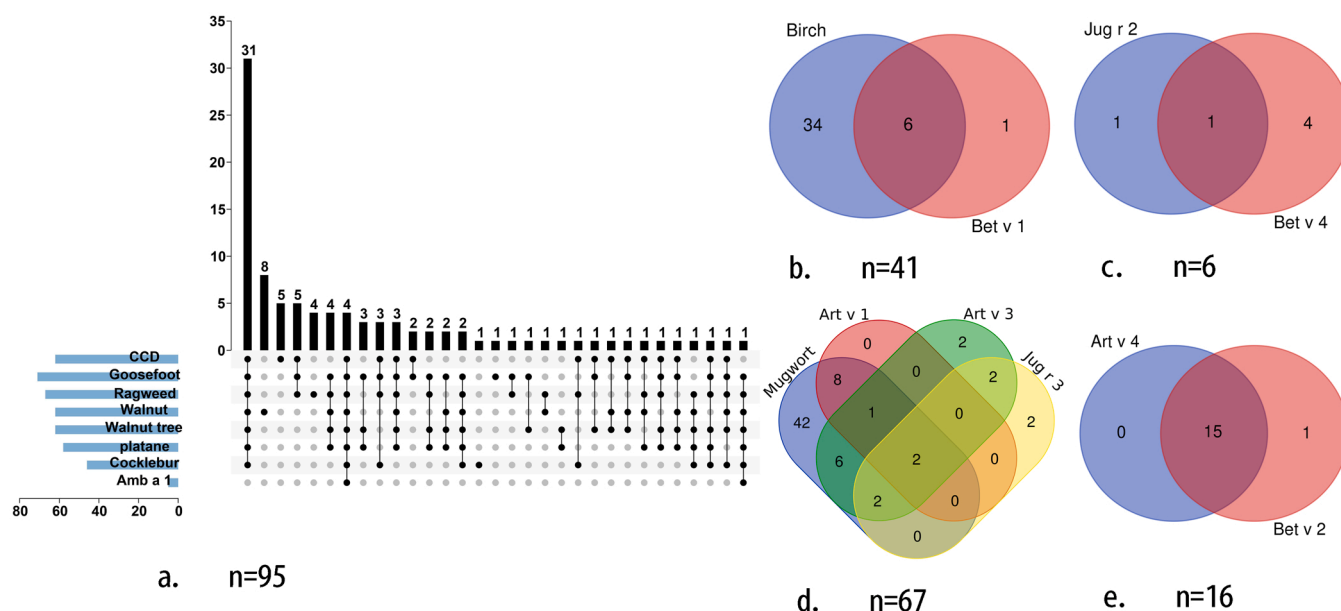


Fig. 4. a–e Venn diagram (b) and UpSet plot (a) of component cosensitization. The Venn diagram or UpSet plot is generated for each cluster according to hierarchical clustering analysis. (a) The connected points in Fig. 4(a) represent the different intersections of elements, analogous to a Venn diagram. Horizontal bars represent the number of elements for each specific comparison, and vertical bars represent the number of elements unique to specific intersections. (b–e) Moreover, the Venn diagram (see description in Fig. 2) numbers display the number of subjects who were cosensitized (or positive) to more than two elements.

pollen. However, the positivity rate of Amb a 1 (7.5%) was not high in the present study. This suggests that these allergens are not allergenic in patients with allergic diseases in southern China.

Bet v 1 is a protein with a molecular weight of 17 kDa that has been identified as the primary allergen of birch in most of Europe and is generally acknowledged to be the main sensitizer and marker allergen of this family. However, Bet v 1 had a sensitization rate of 17.5% in birch-sensitized patients, which was slightly lower than the rate estimated in southern Europe of 40%–65% (Switzerland, Italy) (Cudowska and Kaczmarek, 2004; Rossi et al., 2003). However, Bet v 2 (40.0%) and Bet v 4 (12.5%) in our study showed similar positivity rates to those reported in Italy. Bet v 2 and Bet v 4 are minor birch pollen allergens in Europe (Movérare et al., 2002; Shirasaki et al., 2008). As seen in the Venn diagram, 15 (93.8%) patients were positive for Art v 4 and Bet v 2. Art v 4 and Bet v 2 are panallergens from the profilin family. They have important similarities in sequence and structure, resulting in cross-recognition by IgE antibodies (Offermann et al., 2016; Radauer et al., 2006). Profilin, a functional panallergen, is available as a purified recombinant protein, and may be a useful diagnostic and probably therapeutic reagent (Jeong et al., 2013; Valenta et al., 1992). The lower frequency of Bet v 1 and higher frequency of Bet v 2 reactivity in the patients from southern China might indicate that these subjects have primarily been sensitized to allergens from other sources, such as weed pollen.

Although walnuts are a common food in China and one that may cause a powerful allergic reaction, studies of the walnut components in pollen-sensitized patients are limited. Based on previous studies, Jug r 1 is the best-characterized allergen among the walnut components, and the combination of Jug r 1 and Jug r 4 IgE can optimize diagnosis (Blankstijn et al., 2018; Elizur et al., 2020a). We screened positive walnut samples and recalculated their positive component frequencies as follows: Jug r 3 (12.9%) > Jug r 4 (6.5%) > Jug r 2 (3.2%) > Jug r 1 (1.6%). There was no allergen dominating the response to walnut (*i.e.*, >50%), indicating that most walnut-sensitized patients did not have a walnut allergy. In hierarchical clustering analysis, the third cluster included Bet v 4 and Jug r 2; Bet v 4 (polalcin) is a component of birch pollen (Sekerová and Poláčková, 2011) and Jug r 2 (vicilin seed storage protein) is a component of walnut (Elizur et al., 2020b). We speculated

that the two components were divided into the same cluster in this research due to their extremely low sensitization rate. The Venn diagram results showed that only 1 sample was cosensitized to the two components, thus supporting our conclusion (Fig. 4c). However, experiments with larger sample sizes are required to verify these findings. In hierarchical clustering analysis, the fourth cluster included mugwort, Art v 1, Art v 3, and Jug r 3. The walnut allergen Jug r 3 is a nonspecific lipid transfer protein type 1 (nsLTP1) identified by Pastorello et al. (Dubiel et al., 2019; Pastorello et al., 2004), and previous studies indicated that it is a marker allergen for partial cross-reactivity (Lyons et al., 2021; Skypala et al., 2019). Sarah A. Lyons et al. reported that the odds of a mugwort pollen allergy were nearly four times higher than those of severe walnut allergy. The correlation between Jug r 3 and mugwort ($r = 0.64$) was notable in a study in Athens (Lyons et al., 2021) and higher than that observed in this study ($r = 0.44$). Scala et al. reported a correlation between sensitization to plant food nsLTPs and pollen, and also found that mugwort pollen extracts can inhibit 50–100% IgE binding to food nsLTPs (Ma et al., 2016; Scala et al., 2015). They did not explore the relationship between the presence of cross-reaction and mugwort components. Considering the cross-reactivity of pollen allergens and food allergens, we found a relationship between a pollen allergen component and a food allergen component. Therefore, it could be hypothesized that there are homologous components in pollen and food allergens. If so, we could examine one of the homologous allergen components instead of a chip to reduce the medical expenses in clinical practice (Li et al., 2020).

Despite these promising results, several questions remain. The association between allergen sensitization characteristics and clinical variables such as the patient's degree of control and symptom score, was not assessed. Second, our research lacked cross-inhibition tests; otherwise, more data would be provided regarding the effect of CCD in pollen allergens. To the best of our knowledge, this might be the first report using CRD for sensitization to weed pollen, birch pollen, and food allergens in southern China. Therefore, we could not compare our findings to those of other studies. Future studies on this topic should avoid these limitations.

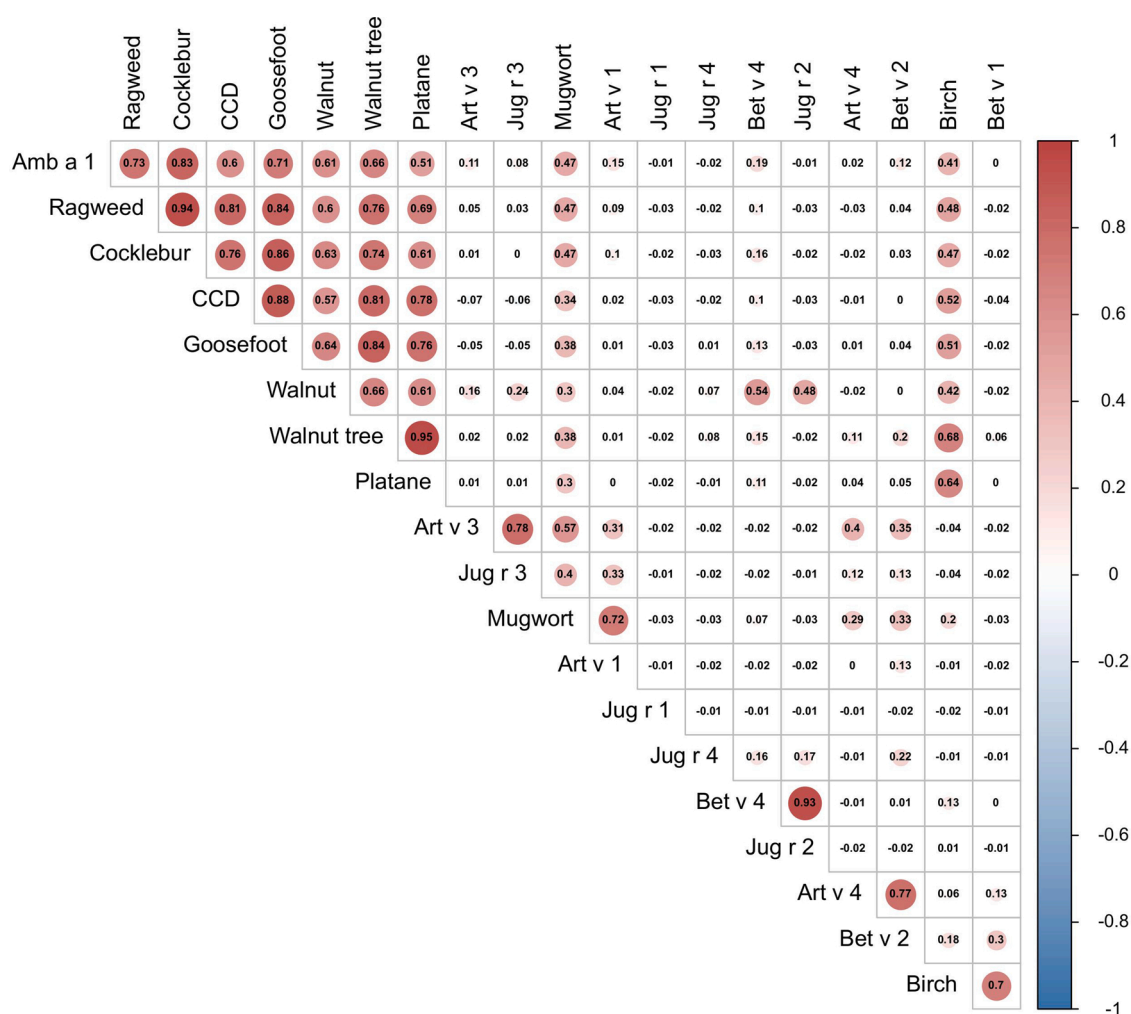


Fig. 5. Correlation relationships between CCD and allergens. Spearman's correlation coefficient (numerical) is shown in square grids. The circles in square grids represent the value of the correlation coefficient (blue indicates a positive correlation, and red indicates a negative correlation). The deeper the colour is, the greater the coefficient.

5. Conclusions

In southern China, the weed pollen (ragweed, cocklebur, and goosefoot) exhibited higher positivity rates in adults and a stronger correlation with CCD. There was a high positivity rate of walnut allergens in patients with mugwort pollen allergies, but the positivity rate of walnut-primary sensitized molecular proteins was not high, suggesting that there may be a cross-reaction between mugwort pollen and walnut. In this study, strong correlations were demonstrated between CCD and pollen crude extracts, and the positivity rate of major pollen allergen components was not high, possibly due to cross-reactivity. However, the relationship between CCD and pollen needs to be further confirmed by a CCD inhibition test.

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Ethics approval

This study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (reference number: GYFYY-2016-73). Written informed consent was obtained from all patients, independently or through their parents (in the case of children), for inclusion in the study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Lina Xu, Wenting Luo, and Yanhong Lu participated in the conception and design of the study, data management, statistical analysis, interpretation of the data and preparation of the manuscript; Xingmei Yu and Chenxi Liao participated in the data collection, data management and preparation of the manuscript; Yinfang Dai, Wenjing Gu and

Zhifeng Huang participated in the statistical analysis, interpretation of the data; Huimin Huang and Peiyan Zheng participated in the data collection, data management; Xinxing Zhang, Hongling Chen and Li Huang participated in the experimental activity and data analysis; Jinping Zheng contributed to the critical revision of the article. Baoqing Sun, Chuangli Hao participated in the conception and design of the study, data analysis, interpretation of the data and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

Declaration of Competing Interest

There are no competing interests.

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Conflicts of interest

The authors declare they have no conflict of interest.

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