

# Three patterns of sensitization to mugwort, timothy, birch and their major allergen components revealed by Latent class analysis

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## ARTICLE INFO

### Keywords:

sIgE  
Mugwort  
Timothy  
Birch  
Latent class analysis

## ABSTRACT

**Background:** Mugwort, timothy, and birch are commonly spread pollen allergens across China. Although several studies have described the rates of sensitization to mugwort, timothy, and birch in China, most of them just on specific whole-allergen extracts but little was known about the co-sensitization characteristics of its allergen components. This study aimed to explore the patterns of sensitization to mugwort, timothy, birch, and their major allergen components.

**Method:** Serum specific IgE (sIgE) levels of allergen components of mugwort, timothy, birch, and cross-reactive carbohydrate determinants (CCD) were detected in 160 patients whose serum showed positive results to at least one of mugwort, timothy, and birch allergens via EUROBlotMaster system. Skin prick testing was utilized to assess the allergic reaction of grass, weed, and tree allergens. Latent class analysis was used to identify underlying patterns of sensitization to a series of allergen components and their corresponding extracts.

**Results:** 88.8% of patients with allergic rhinitis and/or asthma were positive for mugwort-sIgE, 30% for timothy-sIgE, and 32.5% for birch-sIgE. By using the LCA model, three sensitization patterns as “Mugwort, Art v 4, Bet v 2 and Phl p 12 co-sensitized”, “Timothy, mugwort, and CCD co-sensitized”, “Mugwort and Art v 1 co-sensitized” were revealed based on optimal statistical fit in this study. Compared with other clusters, participants in “Mugwort, Art v 4, Bet v 2 and Phl p 12 co-sensitized” pattern were associated with higher sensitization rates of common grass and tree pollens allergen. The spearman's coefficient between CCD and timothy was larger than the corresponding values of CCD with mugwort or birch.

**Conclusion:** CCD and profilin, as minor allergens in pollens, were associated with other pollen sIgE false positives presumably due to cross-reactivity. Patients sensitized with profilin had a significantly higher risk of sensitization to other pollens.

## 1. Introduction

Pollen is an important allergen causing respiratory allergic diseases globally (Cailliot et al., 2017; Davies et al., 2012; Saha et al., 2021). It has been reported that pollen from grass species elicits allergic responses involving 20% of the general population and 40% of atopic individuals,

(Rossi et al., 2008) which result in allergic rhinitis (hay fever) or allergic asthma. Mugwort (*Artemisia vulgaris*), timothy (*Pheluem pretense*) and birch are typical representatives of weed, grass and tree allergens respectively that can provoke allergic rhinitis symptoms.

Mugwort is widely spreading across Europe, North America and parts of Asia, (Himly et al., 2003; Dedic et al., 2009) which is one of the

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main causes of pollinosis in late summer and autumn (Hao et al., 2013; Kapingidza et al., 2019). A cross-sectional study conducted in China has shown that patients with respiratory allergies are commonly sensitized to mugwort, and the reported prevalence is particularly higher in northwest China (58.2%) (Lou et al., 2017). Timothy (*Phleum pratense*) belongs to the Pooideae subfamily, is one of the common sensitized pollens in warm tropics and subtropics, which releases abundant pollens in summer (Visez et al., 2021). Birch pollen is the most dominating tree pollen found in northern China (Hao et al., 2016). A study revealed that the rate of sensitization to birch pollen reached 24.2% in Northwest China (Lou et al., 2017). As a consequence of both the seasonal overlap and the cross-reactivity of species make it difficult to diagnose and treat multi-sensitized to different types of pollen allergy.

Routine diagnosis of allergy to pollen commonly relies on the use of whole-allergen extracts offered for a broad range of species. Extracts are sensitive but cannot further specify the molecular basis of sensitization and explain the origin of polysensitization. Recently, a growing number of recombinant or purified natural allergens from pollen have become available for routine assessment of specific serum IgE. Component-resolved diagnosis (CRD) can help to establish IgE sensitization profiles and potential risks, as well as determine whether specific IgE is the result of primary sensitization or cross-reactivity. This method is especially suitable for those who are polysensitized, either have an unclear symptom and/or sensitization pattern, nor do not respond to their treatment (Sastre et al., 2012; Stringari et al., 2014). Understanding the molecular mechanism of poly-sensitization also helps estimate clinical relevance, especially in those with unexpected sensitization to many pollens.

In Europe, IgE cross-reactivity toward birch pollen allergens and non-tree allergens such as grass, mugwort, and/or olive tree has also been identified (Wolbing et al., 2017; Gurlek et al., 2018; Santos and Van Ree, 2011). This broad cross-reactivity is primarily due to homology among allergens of the profilin and polcalcin protein families and CCD (Santos and Van Ree, 2011; Wopfner et al., 2007; Asam et al., 2015). However, few studies focused on the molecules of pollen allergens in China, moreover, the corresponding data regarding cross-reaction of sensitization to pollen components was still quite limited. Moreover, mugwort had been used in immunotherapy in some parts of China, but CRD had rarely been applied to establish sensitization profiles in China (Gao et al., 2019). Therefore, it was deeply necessary to explore the underlying cross-reactivity and sensitization profiles of different species of allergens in China.

Latent Class Analysis (LCA) is a powerful tool to classify cases using statistical and clinical parameters to choose the 'optimal' model. It was recently used to predict Asthma Exacerbation (Grunwell et al., 2020) and classify AD trajectories regarding genetic and environmental pathomechanisms (Paternoster et al., 2018).

In this study, CRD technology was utilized to detect the serum sIgE of birch, mugwort, timothy allergen components simultaneously among 160 patients, then Using LCA to identify distinct phenotypes in allergic patients based upon molecular parameters of CRD. The study aimed to investigate individual sensitization patterns and identify the putative primary sensitizer, as well as provide guidance for clinical diagnosis and select proper allergen-specific immunotherapy (SIT).

## 2. Methods

### 2.1. Patients and study design

This multi-center epidemiology investigation was conducted in nine medical centers of China from September 2019 to December 2020. Of 160 patients who both met the selection criteria (1) allergic rhinitis and/or asthma participants who were positive to mugwort, birch, or timothy in EUROBlotMaster system (sIgE > 0.35 kU/L); (2) participants who completed with sIgE and SPT detection. The diagnosis of asthma and rhinitis was respectively based on guidelines of GINA (Anon, 2020),

ARIA (Bousquet et al., 2020). Exclusion criteria were as follows: (i) The patients who underwent systemic steroids or biological treatment, malignancy therapy; and (ii) with severe autoimmune diseases, parasitic infections, cancer; and (iii) the subjects with serious skin damage or recent medication were also excluded since the skin damage could interfere with the results of skin prick tests (SPT). All patients gave written informed consent.

In this study, the study sample size was determined based on a similar previously reported study (Liao et al., 2020) in China. Besides, by the sample size procedure for cross-sectional design in SAS9.4 (Copyright 2002–2012 by SAS Institute Inc., Cary, NC, US), the power analysis was additionally performed to evaluate the study sample size justification, we observed that the current sample size ( $n = 160$ ) is sufficient to obtain robust findings since the power was more than 0.8 depending on the detected smaller Spearman's correlation coefficient (Supplemental Figure1).

### 2.2. Detection of pollen-specific IgEs

The tested serum samples of patients were centrifuged for 10 min at 3000 rpm and kept in a  $-80^{\circ}\text{C}$  refrigerator for long-term storage in AIR-SKLRD. IgE responses against 15 different allergens from mugwort, timothy, birch and their allergen component (Art v 1, 3, 4), (Phl p 1, 4, 5, 7, 12), (Bet v 1, 2, 4), and CCD were evaluated in vitro by EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, SH, Germany) according to the instructions provided by the manufacturer. Levels of sIgE were expressed in kU/L, and results of sIgE class based on RAST classification were defined as follows: class 0: < 0.35 kU/L; class 1:  $\geq 0.35$  to < 0.70 kU/L; class 2:  $\geq 0.70$  to < 3.50 kU/L; class 3:  $\geq 3.50$  to < 17.50 kU/L; class 4:  $\geq 17.50$  to < 50.00 kU/L; class 5:  $\geq 50.00$  to < 100.00 kU/L; and class 6:  $\geq 100.00$  kU/L.

### 2.3. Skin prick testing

All patients underwent SPT to three groups (12 kinds) of allergens including trees (*Betula verrucosa*; *Populus alba*; *Ulmus campestris*; *Salix fragilis*; *Platanus hispanica*), weeds (*Ambrosia elatior*; *Artemisia vulgaris*; *Chenopodium album*; *Brassica napus*), grass (*Triticum aestivum*; *Phragmites communis*; *Phleum pratense*); SPTs were performed in the allergy clinics by experienced nurses using commercial allergen extracts (ALK-Abelló Laboratory, Madrid, Spain). Histamine and diluent were used as positive and negative controls. The wheal reaction after 15 min was measured as the mean of the longest diameter and the length of the perpendicular line through its middle. A positive skin reaction was defined as a wheal size  $\geq 3$  mm, after subtraction of the negative control. The result was also expressed as skin index (SI = mean size of allergen wheal/mean size of histamine wheal).

### 2.4. Statistics analysis

Age was presented with median (1st quartile, 3rd quartile) as their distribution is skewed, and the difference of groups was compared by the Kruskal-Wallis H test. Additionally, category variables in this study were depicted as frequency (proportion), and chi-square test was utilized to perform groups comparison. Venn diagram was drawn to investigate the co-sensitization of major pollen allergen. Spearman correlation analysis was used to explore the relationship of sIgE levels between various molecular allergens.

LCA helps to identify subgroups of people who share common characteristics in such a way that people within the subgroups have a similar scoring pattern on the measured variables, while the difference in scoring patterns between the subgroups are as distinctly different as possible. LCA uses a mixture of distributions to identify the most likely model describing the heterogeneity of data as a finite number of classes; We used LCA to investigate the potential patterns of sensitization to mugwort, timothy, birch and their allergen components as well as CCD.

The optimal model of LCA was determined according to the lowest BIC criteria.

All data management and analysis were implemented by SAS9.4 (Copyright 2002–2012 by SAS Institute Inc., Cary, NC, US), as well as the figures were drawn by R studio1.2.5001 (Copyright 2009–2018 R-studio, Inc.).  $P < 0.05$  with two sides was recognized as significant.

### 3. Results

#### 3.1. Clinical characteristics of study patients

In this study, a total of 160 participants were recruited from three areas of China: 14 from east of China, 50 from southwest China, and 96 from northwest China. The median (1st quartile, 3rd quartile) age of patients was 10 (6.0, 28.5) years, and 54.4% of them were men. 58.1% of participants were diagnosed with rhinitis, while 10.6% of patients were suffering from asthma only. Additionally, 31.3% of patients have a comorbidity of rhinitis and asthma.

As shown in Table 1, 142 patients were positive to mugwort, and 48 patients to timothy, as well as 52 patients to birch and 22 patients to CCD. The vast majority of the patients ( $n = 80$ , 50%) were mono-sensitization to mugwort whereas co-sensitization to mugwort, timothy and birch was observed in only 21 (13.1%) patients. Moreover, 10 (47.6%) patients who co-sensitized to mugwort, birch, timothy were positive for CCD-sIgE reactivity (Fig. 1). No CCD IgE was found in patients with isolated birch pollinosis.

#### 3.2. The molecular sensitization patterns of mugwort, timothy and birch

The results revealed that the most common allergen molecule of mugwort was Art v 1 (53.8%). Moreover, it can be observed that Phl p 1 (33.3%) and Phl p 12 (50.0%) were major allergens of timothy as well as Bet v 1 (44.2%) and Bet v 2 (42.3%) were the major sensitized molecules of birch.

The complicated co-sensitization phenomenon of grass pollen allergen components can be seen in this study. The results showed that among mugwort-sensitized patients, 26.1% of them were positive to timothy, and 21.8% to Phl p 12. Additionally, 29.6% of mugwort-sIgE positive patients sensitized to birch, and the joint sensitization rate of mugwort with Bet v 2 achieved 33.8% in this study. Meanwhile, among timothy-sIgE positive patients, 77.1% of participants were positive to mugwort and 50.0% of them to birch.

Our finding also suggests that 80.8% of patients were sensitized to mugwort and 46.2% sensitized to timothy among birch-sIgE positive participants ( $n = 52$ ). In this population, 48.1% of patients were positive to Art v 3, and 32.7% to Phl p 12.

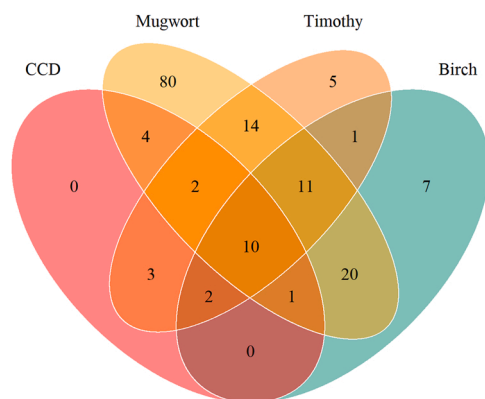


Fig. 1. The pattern of co-sensitization to mugwort, birch, timothy and CCD revealed by the Venn Diagram. Shown are the numbers of co-sensitized patients.

#### 3.3. The correlation of sIgE classes between different allergen components and CCD

The sIgE classes of grass pollen allergens were assessed by the ranked data range from 0 to 6. The results (Fig. 2) show that CCD was significant negatively associated with Art v 1 as well as positively with timothy and Phl p 4 ( $P < 0.01$ ). Spearman correlation analysis shows that Art v 4 had a higher correlation with Phl p 12 ( $r_s = 0.85$ ,  $P < 0.01$ ) and Bet v 2 ( $r_s = 0.92$ ,  $P < 0.01$ ). Moreover, it also can be observed that a strong correlation between Phl p 12 and Bet v 2 ( $r_s = 0.82$ ,  $P < 0.01$ ). We analyzed the positive crossover of Phl p 12, Bet v 2 and Art v 4 based on the results of strong correlation. 34 (62.9%) cases were positive for all three components, and 44 (81.5%) cases reacted to two or more components (Fig. 3).

Although the spearman's correlation coefficient between Phl p 7 and Bet v 4 achieved 1, limited participants were sensitized to Phl p 7 ( $n = 4$ ) and Bet v 4 ( $n = 4$ ) in this study.

Consistent with the results of Table 1 which suggest that Art v 1, Phl p 1 and Bet v 1 are the major allergen components of mugwort, timothy and birch respectively. The correlation analysis also proves that there was a moderate correlation between Art v 1 and mugwort ( $r_s = 0.51$ ,  $P < 0.01$ ), Phl p 1 and timothy ( $r_s = 0.50$ ,  $P < 0.01$ ) as well as Bet v 1 and birch ( $r_s = 0.67$ ,  $P < 0.01$ ).

##### 3.3.1. Co-sensitization patterns of major grass pollen allergen components and CCD

Based on the lowest BIC criteria (Table 2), the optimal LCA model with three latent classes was selected using major grass pollen allergen components and CCD. The patterns of co-sensitization to molecules of mugwort, timothy and birch can be labeled as follows: (1) characterized by a high probability of mugwort, Art v 4, Phl p 12 and Bet v 2 sensitization but a lower probability of CCD sensitization (27.58%); (2) characterized by a high probability of timothy, mugwort and CCD sensitization (10.79%); (3) characterized by a high probability of and mugwort, Art v 1 but a lower probability of timothy, CCD sensitization (61.62%). (Fig. 4).

##### 3.3.2. Clinical characteristics and SPT test of major grass pollens among different co-sensitization patterns

Table 3 showed that there are no significant differences in clinical characteristics among different co-sensitization patterns. Nevertheless, compared with class2 and class3 groups, the class1 group is prone to have significantly higher sensitization rates of common grass (G110, G206, G102, G107) and tree pollen allergens (T502, T520, T522, T518, T535).

### 4. Discussion

In this study, we used CRD to identify underlying sensitive patterns in a well-characterized study population with pollinosis and identified three latent classes of sensitization patterns by LCA. Our findings could provide novel insights to distinguish multiple allergen sensitization results of extract-based diagnostic methods among those with pollinosis, and bring huge clinical implications in the practice of diagnosis and therapy of allergy.

Our finding reveals that the prevalence of sensitization to weed pollens achieves the highest level among individual pollen clusters, followed by grass and tree pollens. In comparison with ours, the corresponding rates of sensitization to grass in Italy and French are much higher (Stringari et al., 2014; Burte et al., 2017). The various geographical distribution of the plant species can explain the differences. Additionally, no bromelain-type CCD sensitization can be detected in isolated birch pollen, and a similar result is also found by Ebo DG (Ebo et al., 2004), which suggests that the major birch pollen allergen lack or have less abundant sugar moieties compared with grass and weed pollens.

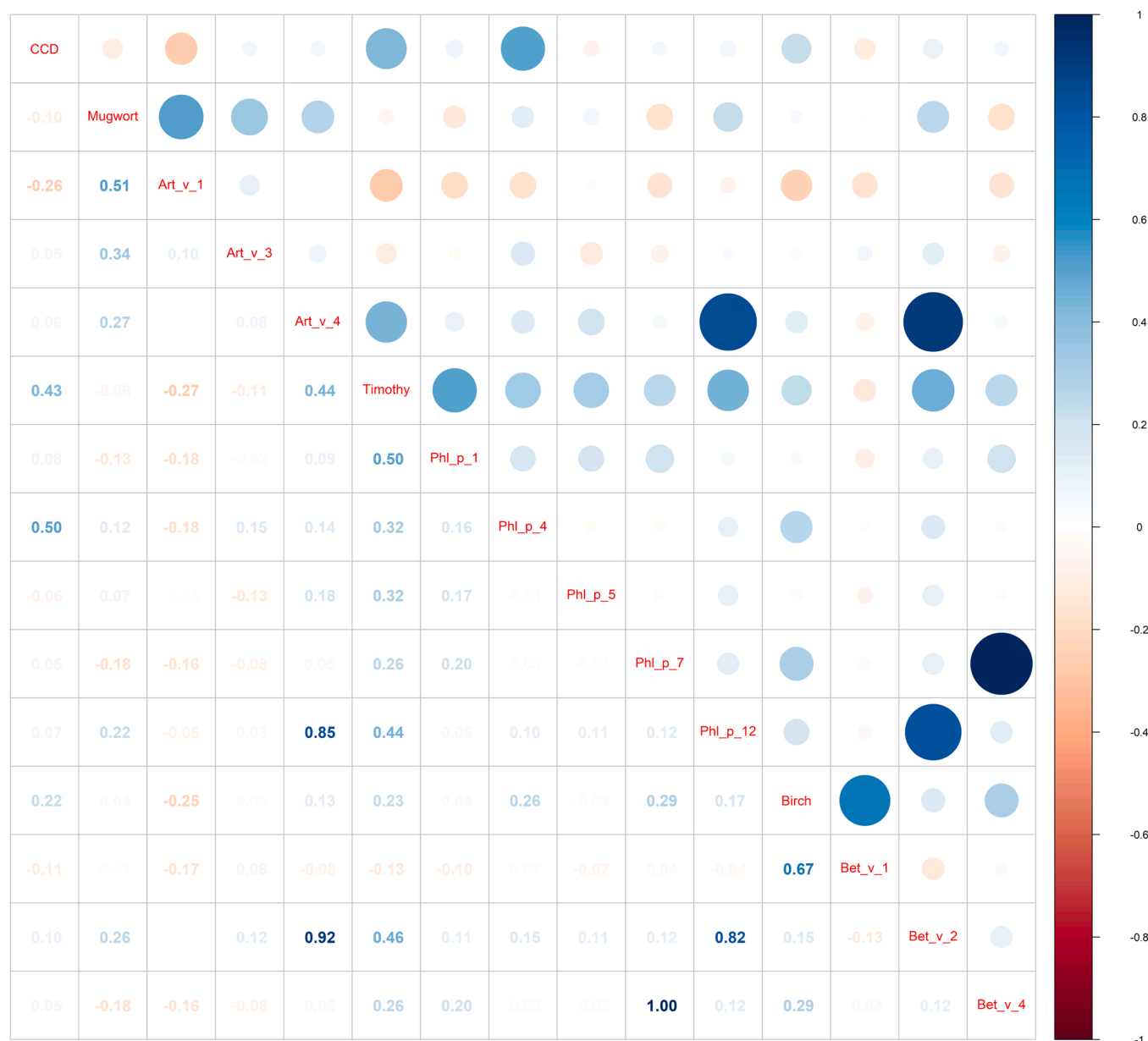


Fig. 2. Spearman Correlation analysis of sIgE classes between different allergen Components and CCD. The depth of the color between two allergens represented the degree of relationship between them. The deeper of the color, the more relevant it is.

It can be observed that Art v 1 and Art v 3 are the most frequently recognized component allergens of mugwort while Art v 4 is comparatively lower in this study. Similar results have also been revealed by others in Mediterranean area (Lombardero et al., 2004) and Northern China (Fu et al., 2018). Previous studies have reported that about 80–95% of mugwort sIgE positive patients sensitized to Art v 1 (Asam et al., 2015; Leb et al., 2008; Jimeno et al., 2004) and 35–85% to Art v 3, (Lombardero et al., 2004; Garcia-Selles et al., 2002) however, those values are higher than us. Notably, the present study reports that Art v 4 is the major component that co-sensitization to Bet v 2 and Phl p 12 in class1 revealed by LCA. We speculate that the lower rate of genuine allergen component of mugwort (Art v 1, Art v 3) might implicate that cross-reactivity caused by profilin. Our findings reveal that the highest sensitization rate is found in Phl p 12 components (50%) among those who are positive to timothy, which over Phl p 1 and Phl p 5, the most predominant grass pollen allergens of timothy (Niederberger et al., 1998). Additionally, 35.4% of patients with timothy sIgE positive are also positive for CCD sIgE. Taken together, we suspect that sensitization

to CCD or profilins is responsible for lower positive responses to Phl p 1 and Phl p 5 in this study. Similarly, there is 44.2% of patients with sensitization to major allergens Bet v 1, but (42.3%) equivalent quantity of birch sIgE positive patients are sensitized to minor allergens Bet v 2. These results reflected the fact that some birch sIgE positive results may suggest probable cross-reactivity other than "genuine" sensitization. The cross-reactive profiles among birch, mugwort and timothy were further verified by LCA.

Class 1 represented an almost high prevalence of mugwort and Art v 4, Phl p 12, Bet v 2, with low CCD. Art v 4, Phl p 12, Bet v 2 are representative of profilin (Rodriguez Del Rio et al., 2018), a ubiquitous family of proteins that control actin polymerization in eukaryotic cells (Valenta et al., 1992; Mares-Mejia et al., 2020), and the Venn diagram (Fig. 3) showed a high degree of overlap (62.9%). In accordance with others (Hirschwehr et al., 1998), our data confirm that mugwort frequently induces sensitization to profilin. Moreover, double-sensitization or polysensitization is associated with a unique binding pattern characterized by a strikingly high prevalence of profilin

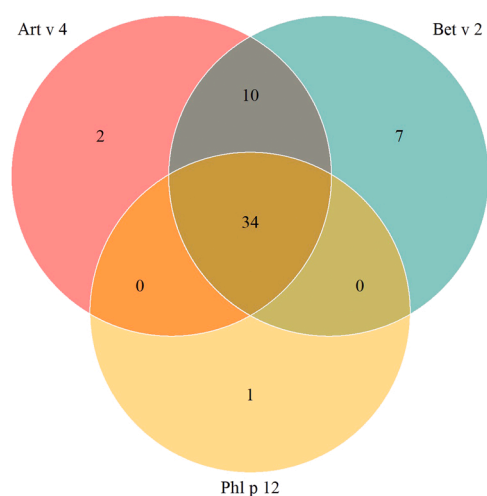


**Fig. 3.** The pattern of co-sensitization to Phl p 12, Bet v 2 and Art v 4 revealed by the Venn Diagram. Shown are the numbers of co-sensitized patients.

in Class 1. As a result of a conserved structure and 80% identical sequence of amino acids, indicating a high degree of cross-reactivity even from distantly related species (Chruszcz et al., 2018), consequently, we can see that class 1 shows positive skin tests for various types of pollen in Table 3.

17 (10.74%) participants are included in latent class 2 which is characterized by a high probability of timothy, mugwort and CCD co-sensitization. The prevalence of CCD is significantly higher in class 2 than classes 1 and 3. The correlation between CCD and mugwort or its components is poor or even negative, by contrast, Phl p 4 had a strong correlation with CCD. It means that sensitization to bromelain-type CCD generally originates from timothy pollens, and less from weed pollens in China. Phl p 4, a glucose dehydrogenase member of the berberine bridge

enzyme-like enzyme family, can be glycosylated at 4 sites by CCD, (Zafred et al., 2013) thus resulting in co-sensitization of CCD and Phl p 4. It has been well established that CCDs are the broadest and most common causes of cross-reactivity among natural allergen sources and molecules (Ward et al., 2014). Accordingly, CCD-specific IgE plays a confounding effect on the cross-reactivity of birch, mugwort and timothy. Moreover, CCD can cause extensive cross-reactivity between different allergen sources (Table 3). It is difficult to identify disease-eliciting pollen sources by means of extract-based skin SPT in patients sensitized to multiple pollens.

45 patients (61.2%) are allocated to class 3, which is characterized by a higher prevalence of mugwort and Art v 1, but lower CCD and Art v 4/ Bet v 2/ Phl p 12. It can be observed that numerous skin prick test positive reactions against tree, grass, and weed pollen show a lower positive rate in class3 than class1 or class2, which reflected that genuine pollen polysensitization against birch, grass, and weed pollen without the involvement of the pollen panallergens profilin or CCD in this group.

Totally, the major clinical implications of this study are focusing on the precision diagnosis of allergy and adding knowledge of the complicated molecular mechanism of sensitization to common grass pollens. First, the present study investigated the joint sensitization patterns of whole allergen extracts and allergen components would be beneficial for disclosing the cross-reactivity of similar molecular proteins in different allergens. Second, the comprehensive analysis of the sensitization among common allergen components would be beneficial

**Table 2**  
Model fit statistics of latent class analysis.

| K-class | AIC    | BIC    | Entropy |
|---------|--------|--------|---------|
| 2       | 631.89 | 727.23 | 0.96    |
| 3       | 562.43 | 706.96 | 0.96    |
| 4       | 542.00 | 735.74 | 0.96    |

**Note:** Akaike information criterion, AIC; Bayesian Information Criteria, BIC

**Table 1**  
The clinical and allergy sensitization characteristics of participants.

| Variables              | Total<br>N = 160 | Mugwort<br>N = 142 | Timothy<br>N = 48 | Birch<br>N = 52 | P value |
|------------------------|------------------|--------------------|-------------------|-----------------|---------|
| Age                    | 10.0(6.0,28.5)   | 11.0(6.0,29.0)     | 8.0(6.0,11.0)     | 8.0(6.0,12.0)   | 0.028   |
| Sex                    |                  |                    |                   |                 | 0.462   |
| Woman                  | 73(45.6)         | 65(45.8)           | 19(39.6)          | 19(36.5)        |         |
| Man                    | 87(54.4)         | 77(54.2)           | 29(60.4)          | 33(63.5)        |         |
| Mode of delivery       |                  |                    |                   |                 | 0.375   |
| Normal delivery        | 110(68.8)        | 98(69.0)           | 28(58.3)          | 33(63.5)        |         |
| Cesarean section       | 50(31.3)         | 44(31.0)           | 20(41.7)          | 19(36.5)        |         |
| Ethnic                 |                  |                    |                   |                 | 0.906   |
| Han                    | 137(85.6)        | 121(85.2)          | 42(87.5)          | 44(84.6)        |         |
| Minority               | 23(14.4)         | 21(14.8)           | 6(12.5)           | 8(15.4)         |         |
| Diseases               |                  |                    |                   |                 | 0.656   |
| AR                     | 93(58.1)         | 81(57.0)           | 26(54.2)          | 30(57.7)        |         |
| AR+AS                  | 50(31.3)         | 47(33.1)           | 14(29.2)          | 18(34.6)        |         |
| AS                     | 17(10.6)         | 14(9.9)            | 8(16.7)           | 4(7.7)          |         |
| Allergic sensitization |                  |                    |                   |                 |         |
| CCD                    | 22(13.8)         | 17(12.0)           | 17(35.4)          | 13(25.0)        | 0.001   |
| Mugwort                | 142(88.8)        | 142(100.0)         | 37(77.1)          | 42(80.8)        | < 0.001 |
| Art v 1                | 86(53.8)         | 84(59.2)           | 14(29.2)          | 17(32.7)        | < 0.001 |
| Art v 3                | 66(41.3)         | 62(43.7)           | 18(37.5)          | 25(48.1)        | 0.563   |
| Art v 4                | 46(28.8)         | 42(29.6)           | 28(58.3)          | 20(38.5)        | 0.002   |
| Timothy                | 48(30.0)         | 37(26.1)           | 48(100.0)         | 24(46.2)        | < 0.001 |
| Phl p 1                | 19(11.9)         | 14(9.9)            | 16(33.3)          | 7(13.5)         | < 0.001 |
| Phl p 4                | 5(3.1)           | 4(2.8)             | 5(10.4)           | 5(9.6)          | 0.061   |
| Phl p 5                | 4(2.5)           | 3(2.1)             | 4(8.3)            | 1(1.9)          | 0.094   |
| Phl p 7                | 4(2.5)           | 3(2.1)             | 4(8.3)            | 4(7.7)          | 0.095   |
| Phl p 12               | 35(21.9)         | 31(21.8)           | 24(50.0)          | 17(32.7)        | 0.001   |
| Birch                  | 52(32.5)         | 42(29.6)           | 24(50.0)          | 52(100.0)       | < 0.001 |
| Bet v 1                | 23(14.4)         | 18(12.7)           | 4(8.3)            | 23(44.2)        | < 0.001 |
| Bet v 2                | 51(31.9)         | 48(33.8)           | 31(64.6)          | 22(42.3)        | 0.001   |
| Bet v 4                | 4(2.5)           | 3(2.1)             | 4(8.3)            | 4(7.7)          | 0.095   |

**Note:** AS, allergic asthma; AR, allergic rhinitis

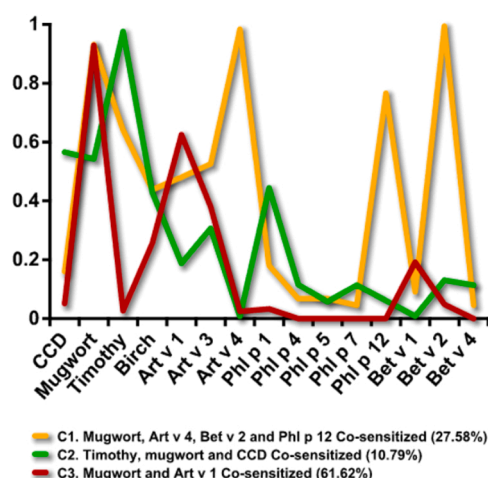


**Fig. 4.** Three-class sensitization patterns are identified by latent class analysis based on allergen components and CCD. Shown are probabilities of positive sIgE responses (y-axis) to the allergen components and CCD (x-axis).

**Table 3**

The characteristics of participants in various latent classes.

| Variables                   | Class 1<br>N = 45 | Class 2<br>N = 17 | Class 3<br>N = 98  | P value |
|-----------------------------|-------------------|-------------------|--------------------|---------|
| Age, years                  | 9.0<br>(6.0,23.0) | 7.0<br>(4.0,11.0) | 12.0<br>(6.0,29.0) | 0.113   |
| Sex, %                      |                   |                   |                    | 0.562   |
| Woman                       | 18(40.0)          | 7(41.2)           | 48(49.0)           |         |
| Man                         | 27(60.0)          | 10(58.8)          | 50(51.0)           |         |
| Mode of delivery, %         |                   |                   |                    | 0.929   |
| Normal delivery             | 31(68.9)          | 11(64.7)          | 68(69.4)           |         |
| Cesarean section            | 14(31.1)          | 6(35.3)           | 30(30.6)           |         |
| Ethnic, %                   |                   |                   |                    | 0.188   |
| Han                         | 37(82.2)          | 17(100.0)         | 83(84.7)           |         |
| Minority                    | 8(17.8)           | 0(0.0)            | 15(15.3)           |         |
| Diseases, %                 |                   |                   |                    | 0.256   |
| AR                          | 29(64.4)          | 7(41.2)           | 57(58.2)           |         |
| AR+AS                       | 11(24.4)          | 6(35.3)           | 33(33.7)           |         |
| AS                          | 5(11.1)           | 4(23.5)           | 8(8.2)             |         |
| Allergy sensitization,<br>% |                   |                   |                    |         |
| W301                        | 31(68.9)          | 8(47.1)           | 58(59.2)           | 0.261   |
| W302                        | 40(88.9)          | 14(82.4)          | 90(91.8)           | 0.464   |
| W305                        | 35(77.8)          | 10(58.8)          | 35(35.7)           | <0.001  |
| T502                        | 27(60.0)          | 1(5.9)            | 34(34.7)           | <0.001  |
| G110                        | 23(51.1)          | 5(29.4)           | 7(7.1)             | <0.001  |
| W310                        | 27(60.0)          | 4(23.5)           | 19(19.4)           | <0.001  |
| G206                        | 20(44.4)          | 6(35.3)           | 8(8.2)             | <0.001  |
| T520                        | 33(73.3)          | 4(23.5)           | 44(44.9)           | <0.001  |
| T522                        | 34(75.6)          | 4(23.5)           | 25(25.5)           | <0.001  |
| G102                        | 40(88.9)          | 7(41.2)           | 24(24.5)           | <0.001  |
| T518                        | 38(84.4)          | 5(29.4)           | 27(27.6)           | <0.001  |
| T524                        | 12(26.7)          | 1(5.9)            | 13(13.3)           | 0.062   |
| T535                        | 27(60.0)          | 4(23.5)           | 33(33.7)           | 0.004   |
| G107                        | 21(46.7)          | 4(23.5)           | 21(21.4)           | 0.007   |

**Note:** *Ambrosia elatior*, W301; *Artemisia vulgaris*, W302; *Chenopodium album*, W305; *Betula verrucosa*, T502; *Pheum pratense*, G110; *Brassica napus*, W310; *Triticum aestivum*, G206; *Ulmus campestris*, T520; *Salix fragilis*, T522; *Cynodon dactylon*, G102; *Populus alba*, T518; *Mediterranean cypress*, T524; *Platanus hispanica*, T535; *Phragmites communis*, G107.

to clinical diagnosis and management in medical practices. Third, the assessment of joint sensitization associations among mugwort, timothy, birch and their major allergen components contributes to the understanding of the development of pathogenesis for IgE-mediated allergy.

Several limitations should appeal to our attention in this study. First, we used the EUROIMMUN to evaluate atopic sensitization although ImmunoCAP was the “gold standard” for sIgE detection which was

recommended by the World Health Organization (Cramer, 2013). However, a study performed in Germany (Skevaki et al., 2021) suggests that ImmunoCAP and Euroline™ panel showed good analytical and diagnostic concordance in the detection of allergy. In addition, compared with ImmunoCAP, the EUROIMMUN system is easy to conduct and inexpensive as well as has been widely used to assess atopic sensitization (Di Fraia et al., 2019; Wongpiyabovorn et al., 2018; Luo et al., 2021). The EUOLine method only requires 150 µL to complete multiple allergens at the same time, whereas the ImmunoCap Singleplex assay needs 40 µL per test allergen. Furthermore, the present study was utilized skin prick tests and specific Immunoglobulin E measurement (sIgE) results revealed by the EUROIMMUN system concurrently for clinical diagnosis of allergy, thus making our results more reliable. Second, we did not explore the relationship between three reactivity profiles and clinical severe status. Although the clinical relevance of IgE against profilin is limited for a long time, (Asero et al., 2010; Canis et al., 2011) many studies have proved that profilin could be a marker of predisposition to many severe allergic diseases (Asero et al., 2015; Ruiz-Hornillos et al., 2020). With the clinical relevance of sensitization to profilin is questionable, analyzing clinical severe symptoms and allergenic molecules of pollens in Chinese could yield interesting results. Third, we discussed only the molecules mainly proposed in an algorithm by LCA, but less taken into account other minor allergenic proteins, such as calcium-binding protein (Bet v 4), which might be responsible for cross-reactivity in a given patient. Nonetheless, around 2.5% of patients who were Bet v 4 sensitized, applying there is a little effect in Chinese.

As far as we know, this is the first study using the LCA method to identify distinct phenotypes in allergic patients based upon molecular parameters of the CRD technology. However, our study has the following implications for clinical practice. Accordingly, approximately 30% of our patients reacted to profilin and 13% reacted to CCD, which are highly cross-reactive and responsible for skin test reactions to many pollen sources. The clinical history and SPT and/or IgE tests with extracts are not sufficient to discriminate the pollen or pollens eliciting symptoms, especially in classes 1 and 2. For improving the specificity of in vitro allergy diagnostics and SIT efficacy in patients who are sensitized to multiple pollens, we should consider CRD diagnosis of allergen as well as integrate with clinical history and SPT responses in clinical practice. For example, it was proposed that patients with SPT reactivity to mugwort pollen extracts should receive SIT only for those who simultaneously sensitization to major allergenic molecules of mugwort (Art v 1), like class 3, since AIT should be applied if the patient demonstrates hypersensitivity to genuine allergens. The clinical relevance of class 2 could be that the timothy -positive patients should use CCD inhibitor in clinical practice before receiving timothy immunotherapy, to reduce the false positive interference caused by CCD. In China, profilins were believed as the main contributors to clinically irrelevant positive pollen extracts results than CCD. Lastly, further studies should be performed to determine whether profilin can serve as a marker to predict the severity of allergic diseases.

In conclusion, CRD with currently available pollen allergens can effectively illuminate the molecules against major and minor allergens who are sensitized to birch, mugwort, timothy and found three groups of patients differ in their distribution patterns of molecular allergens. Assessment of sensitization to individual allergen components would be beneficial for the improvement of the specificity diagnostics of allergy.

### Ethics approval and consent to participate

This study involving human participants was reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYFY-2018-93), and all experiments were conducted following the relevant guidelines and regulations of the Ethics Committee. The patients/participants [legal guardian] provided written informed consent to participate in this study.

## Funding

This study was funded by the Guangzhou Science and Technology Foundation, China (NO: 202102010327), SKLRD, China (NO: Z-2022-09) and National Natural Science Foundation of China (NO: 81871736), and the First Affiliated Hospital of Guangzhou Medical University, China (ZH201904).

## CRediT authorship contribution statement

Liting Wu, Xiangqing Hou, Wenting Luo, Baoqing Sun and Chen Huang designed the study. Liting Wu, Wenting Luo, Haisheng Hu, Xianhui Zheng, Yuemin Chen, Zhangkai J Cheng completed the data management and data cleaning as well as interpretation. Xiangqing Hou and Liting Wu performed the statistical analysis. Liting Wu and Xiangqing Hou, Wenting Luo drafted the manuscript. Chen Huang, Baoqing Sun edited the manuscript. All authors contributed to the critically revision of the manuscript and approved the final version.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author (email: sunbaoqing@vip.163.com) on reasonable request.

## Acknowledgements

Thanks the Biobank for Respiratory Diseases in the National Clinical Research Center for Respiratory Disease (BRD-NCRCRD, Guangzhou, Southern China). We would like to thank everyone involved in the collection, detection, and date management of the serum samples.

## Consent for publication

The funders had no roles in study design, data collection, data analysis, interpretation and writing of the report. Baoqing Sun and Xiaohua Douglas Zhang had full access to the data in the study and had final responsibility for the decision to submit for publication.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.molimm.2022.03.009](https://doi.org/10.1016/j.molimm.2022.03.009).

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