

Identifying Potential Co-Sensitization and Cross-Reactivity Patterns Based on Component-Resolved Diagnosis

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Keywords

Component-resolved diagnosis · Allergy rhinitis · Asthma · Dust mite sensitized · Co-Sensitization · Cross-Reactivity

Abstract

Background: Component-Resolved diagnosis (CRD) can help to establish immunoglobulin E (IgE) sensitization profiles and potential risks and determines whether specific IgE is the result of primary sensitization or cross-reactivity, especially for those who are polysensitized. **Methods:** We recruited 432 patients with mite-sensitized respiratory allergic diseases to study the co-sensitization and cross-reactivity of the 17 allergen components in Guangdong Province, China, using the CRD method and to describe the potential association between allergen components. **Results:** Among the 432 patients, serum specific immunoglobulin E of the 17 components were tested by EUROIMMUN system. Der *p* 1 (81.48%), Der *f* 2 (77.78%), Der *f* 1 (74.07%), Der *p* 2 (66.20%) and Der *p* 23 (54.63%) were the main sensitized components in patients with mite-sensitized respiratory allergy, while the components of cockroach, crab, and shrimp had a lower positive rate. In the crude extract allergen-positive samples, Der *f* 2 (91.06%) and Der *f* 1 (86.72%) were the major sensitized

components of Der *f*, while Der *p* 1 (94.52%), Der *p* 2 (78.36%), Der *p* 23 (63.29%) were the major sensitized components of Der *p*. And other components of Der *p* such as Der *p* 7 (34.25%), Der *p* 5 (17.81%), Der *p* 10 (12.05%), Der *p* 3 (1.92%) were all below 50.00%. Blo *t* 5 (54.55%) was one of the major components of Blo *t*. The positive rates of all Bla *g* components were as follows, rBla *g* 2 (15.56%) > rBla *g* 5 (8.89%) > rBla *g* 4 (4.44%) > rBla *g* 1 (1.11%). The positive rate of the only available pen *a* 1 component was 9.43%. Using hierarchical cluster and optimal scale analysis, 17 components can be roughly divided into 5 different sensitization clusters. Also, from the results of the Venn diagram, the allergen component in each cluster has a high proportion of co-sensitization and cross-reactivity. Regardless of age, total IgE levels, and disease type factors, similar sensitization profiles were observed for each component in the same category based on hierarchical clustering analysis. **Conclusions:** Epidemiological data on allergen components causing allergic symptoms can be further understood using CRD. Der *p* 1, Der *p* 2, Der *p* 23, Der *f* 1, Der *f* 2 as the primary sensitizing component

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of the study cohort. The positive rate for Blo t 5 was 28.01% for all populations and 54.45% for Blo t-positive samples. In addition, CRD allows us to identify more potential allergen associations such as common sensitivities and cross-reactions between component proteins. Based on these results, we suggest that when patients are identified as sensitized to a particular allergen, clinicians can pay more attention to other allergy components that are closely related to it.

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Introduction

Allergic rhinitis (AR) and asthma (AS), the most common respiratory allergic diseases, are global health problems that cause major illness and disability worldwide and affect an individual's social life, sleep pattern, school activities, and daily work [1]. The prevalence of self-reported AR has been estimated to be approximately 25% in children and >40% in adults [2, 3]. The hallmark of the immune response in allergic patients is the ongoing production of allergen-specific immunoglobulin E (sIgE). This is defined as sensitization and is considered necessary in detecting allergies [4]. Currently, the natural allergen extracts are used in the establishment of the clinical diagnosis of allergy, but the extracts are usually heterogeneous because they may include not only the major allergens but also the cross-reactive allergens, non-allergenic antigens, and interfering substances [5, 6]. Moreover, it is difficult to achieve standardization in the extracts; hence, the clinical symptoms of allergic diseases are poorly correlated [7].

Advancements in the field of recombinant allergens caused by the application of deoxyribonucleic acid technology in the late 1980s led to the development of the following new concept in the diagnosis of allergy: component-resolved diagnosis (CRD) [8]. CRD can help to establish immunoglobulin E (IgE) sensitization profiles and potential risks and determines whether specific IgE is the result of primary sensitization or cross-reactivity, especially for those who are polysensitized, have an unclear symptom and/or sensitization pattern, or who do not respond to their treatment [9, 10]. The World Allergy Organization-AR and its Impact on Asthma-Global Allergy and Asthma European Network consensus has documented that CRD should be included as a second-line diagnostic method for AR or AS [11]. In developed countries such as Europe and the United States, CRD has been widely used in the diagnosis and treatment of allergic diseases, but the understanding and research on CRD in China is still in its infancy.

EUROIMMUN system (EUROIMMUN, Oumeng Medical Laboratory, Lübeck, Germany) is based on the principle of immunoblotting using 150 µL of serum to detect the specific IgE of the 17 component proteins. In the present study, we recruited a group of patients with mite-sensitized respiratory allergic diseases to study the co-sensitization and cross-reactivity of the 17 allergen components in Guangdong Province, China, using the CRD method and to describe the potential association between allergen components.

Materials and Methods

Serum Sample

The Allergy Information Repository of the State Key Laboratory of Respiratory Disease (AIR-SKLRD) is a large serum bank established inside the Guangzhou Institute of Respiratory Hospital. Since the last decade, AIR-SKLRD has already started to collect and store serum samples from patients with allergic diseases, together with their clinical information [6, 12].

Patients' sera were randomly obtained from the serum bank of AIR-SKLRD, and all of these patients have been diagnosed to have AS and/or AR based on the Global Initiative for Asthma [13] and AR and its Impact on Asthma [1] guidelines and have the following characteristics: (a) have a positive ImmunoCAP serum sIgE antibody against at least one of the dust mite extracts (including Der p, Der f, Blo t; ≥ 0.35 kU/L) and (b) show a positive result in the bronchial provocation test or bronchial dilation test. Patients with one of the following characteristics were excluded: those with incomplete medical record, those who had lost contact, those who failed to provide informed consent, those with identified immunodeficiency, those who were currently on immunotherapy or immunomodulatory agent treatment, those who had parasitic infections, and those who verbally stated that they did not receive any treatment or drug prescription prior to serum collection to minimize the interference of the laboratory findings. All serum samples not meeting the inclusion criteria were excluded from this study.

Finally, a total of 432 patients were enrolled in this study, and there were 279 males and 153 females. The age distribution was 12 (7, 31; median [interquartile range]), with 116 patients having AR only, 177 patients having AS only, and 139 patients having AR + AS. The patients' basic information is shown in Table 1.

Measurement of Allergen-sIgE

Sera sIgE to Der f, Der f 1, Der f 2, Der p, Der p 1, Der p 2, Der p 3, Der p 5, Der p 7, Der p 10, Der p 23, Blo t, Blo t 5, Pen a 1, Eri s 1, rBla g 1, rBla g 2, rBla g 4, rBla g 5, and Per a 7 were measured using the EUROIMMUN system according to the manufacturer's instructions. sIgE levels were expressed in international unit per milliliter (IU/mL) with the following range: 0.35–100 IU/mL. Any measurement over the upper limit of the detected range was given a value of 100 IU/mL. Tests with sIgE level <0.35 IU/mL were defined as sIgE-negative and ≥ 0.35 IU/mL were defined as sIgE-positive. sIgE-positive tests were categorized into the following 6 classes: class 1 (≥ 0.35 to <0.70 IU/mL), class 2 (≥ 0.70 to <3.50 IU/mL), class 3 (≥ 3.50 to <17.50 IU/mL), class 4 (≥ 17.50 to <50 IU/mL), class 5 (≥ 50 to <100 IU/mL), and class 6 (≥ 100 IU/mL).

Table 1. The sIgE concentrations of patients sensitized to Der *p*, Der *f*, and Blo *t* based on disease and demographic details

	Number of patients	Median (IQR)			
		tIgE	Der <i>p</i>	Der <i>f</i>	Blo <i>t</i>
Total	432	435 (196–880)	30.80 (3.39–91.98)	37.30 (5.54–90.40)	3.86 (1.02–17.80)
Gender					
Male	64.58 (279)	529 (233–981)	41.50 (5.77–100)	60.60 (12.70–100)	4.65 (1.46–18.10)
Female	35.42 (153)	272 (121–667)	13.70 (1.60–73.25)	20.80 (2.34–77.80)	2.07 (0.61–12.17)
Age, years old					
<12	53.70 (232)	584 (234–1028)	65.25 (15.38–100)	81.10 (32.70–100)	5.85 (1.78,22.80)
12–18	9.26 (40)	472 (270–977)	56.00 (28.03–100)	66.65 (28.03–81.10)	4.70 (1.30–11.70)
≥18	37.04 (160)	253 (113–600)	4.42 (1.17–25.33)	7.82 (1.19–30.30)	2.18 (0.55–3.33)
Diagnosis					
Patients with AR	26.85 (116)	253 (107–526)	21.60 (4.08–58.58)	20.80 (4.36–64.00)	1.02 (0.56–2.73)
Patients with AS	40.97 (177)	469 (205–808)	17.00 (1.30–77.50)	21.65 (2.11–77.50)	3.69 (1.64–6.95)
Patients with AR and AS	32.18 (139)	680 (242–1167)	64.35 (17.60–100)	76.5 (29.90–100)	7.93 (2.45–29.00)

Among the 432 patients, all patients were tested for total IgE, but not all sIgE of Der *p*, Der *f*, and Blo *t* was detected as we only ensured that all patients were serologically sensitized to at least one of them.

AR, allergic rhinitis; AS, asthma; sIgE, specific immunoglobulin E; IQR, interquartile range.

Ethical Approval

This study and the use of the human serum samples were approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYYY-2016-73). All patients, independently or through their parents (in the case of children), provided their written informed consent for inclusion in the study. A 5 mL of venous blood was collected from each patient, centrifuged at 1,000 g for 10 min, and stored at –80 °C until use.

Data Analysis

Statistical studies were performed using Excel 2016 (Microsoft® Excel® 2016) and Statistical Package for the Social Sciences 22.0 (International Business Machines Corporation Corp., Armonk, NY, USA). Parametric quantitative data were presented as the mean ± SD. Nonparametric quantitative data were presented as the median (interquartile range). A hierarchical cluster test was used to classify all variables by analyzing the similarity or dissimilarity of the data, and the optimal scale analysis was used to verify the correlation between allergen components. Categorical data were reported as a percentage showing the proportion of positive results. Proportions were compared between the groups using chi-square test, and *f* test was used to compare the variance of data among the groups. *p* value <0.05 was considered to be statistically significant.

Results

The Distribution of EUROIMMUN-Identified Components in 432 Polysensitized Respiratory Allergic Patients

Among the 432 patients, serum sIgE of the 17 components were tested (Fig. 1a). Der *p* 1 (81.48%), Der *f* 2 (77.78%), Der *f* 1 (74.07%), Der *p* 2 (66.20%) and Der *p*

23 (54.63%) were the main sensitized components in patients with mite-sensitized respiratory allergy, while the components of cockroach, crab, and shrimp had a lower positive rate. For all included population (mite-allergy), all components of Bla *g* and Per *a* have very low sensitization rates (both below 10%), and it seems that rBla *g* 2 and rBla *g* 5 are relatively important components. Since we did not measure the sIgE reactivity of shrimp and crab crude extract, we can only calculate the positive rates of Eri *s* 1 and Pen *a* 1 for all patient populations, 10.42 and 4.17% respectively.

In Figure 1b we show the sensitization frequency of the components in the crude extract allergen-positive samples to distinguish which are the dominant component proteins. Der *f* 2 (91.06%) and Der *f* 1 (86.72%) were the major sensitized components of Der *f*, while Der *p* 1 (94.52%), Der *p* 2 (78.36%), Der *p* 23 (63.29%) were the major sensitized components of Der *p*. And other components of Der *p* such as Der *p* 7 (34.25%), Der *p* 5 (17.81%), Der *p* 10 (12.05%), Der *p* 3 (1.92%) were all below 50.00%. Blo *t* 5 (54.55%) was one of the major components of Blo *t*. The positive rates of all Bla *g* components were as follows, rBla *g* 2 (15.56%) > rBla *g* 5 (8.89%) > rBla *g* 4 (4.44%) > rBla *g* 1 (1.11%). The positive rate of the only available pen *a* 1 component was 9.43%.

Furthermore, we observed from Figure 1a that the major components of the Der *p* and Der *f* are positive at high sIgE levels (4th and 5th class). For example, the percentage of samples with sIgE titer at level 5 of Der *f* 2 was

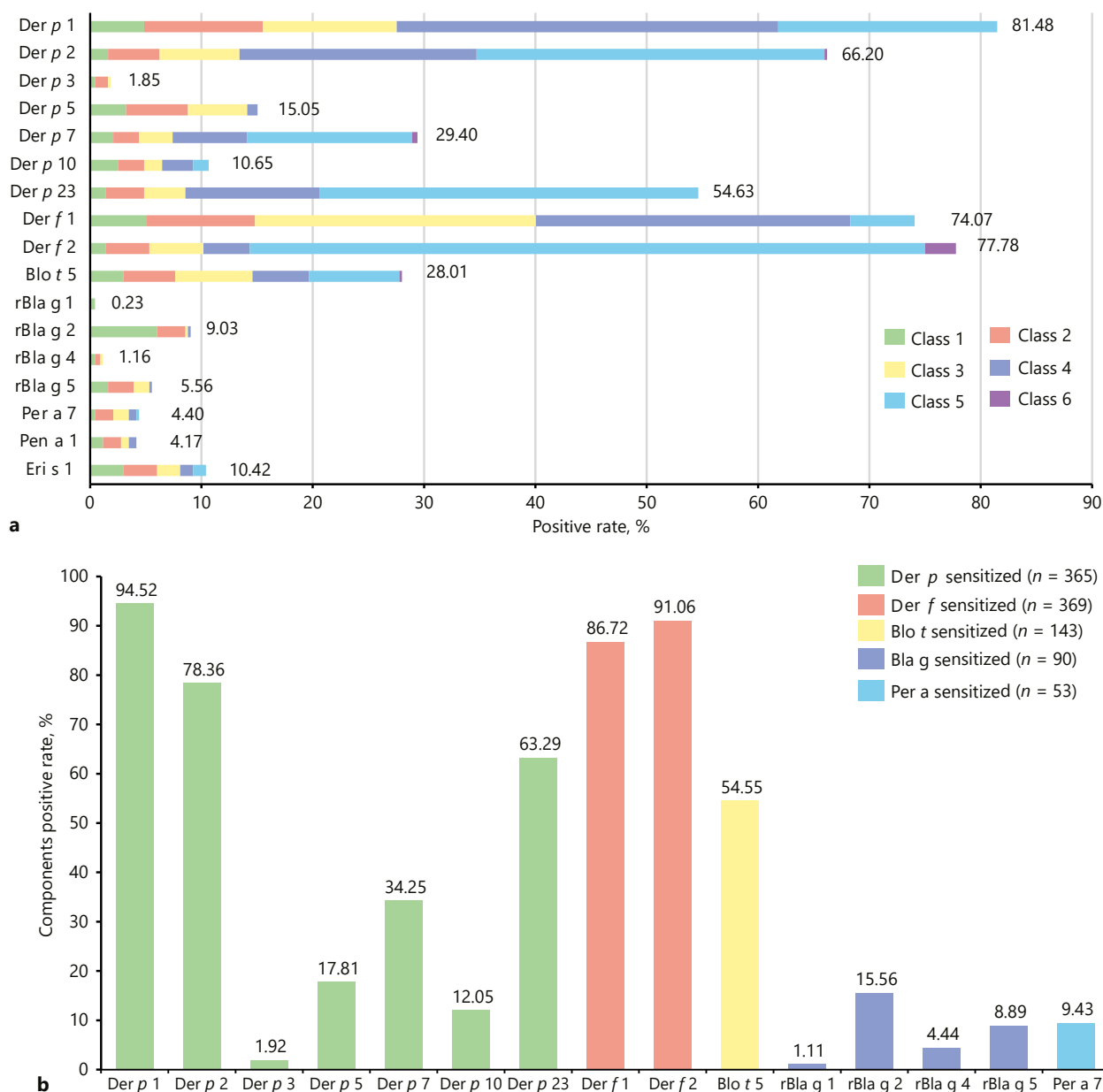


Fig. 1. a The distribution of positive rates of the 17 component allergens in all enrolled patients and **(b)** the components sensitized frequency in only the corresponding crude extract sensitized subjects. All the 432 patients were positive for at least one of the dust mite crude extract allergens (detected by ImmunoCAP), and all patients were tested for all the 17 component allergens using the EUROIMMUN system.

77.98% (262/336), and the percentage of samples with sIgE titer at level 5 of Der p 23 was 62.29% (147/236). In Der p 1 positive samples, the proportion of sIgE titers in grades 5 and 4 was 56.82% (200/352), while Der p 2 was 79.37% (227/286).

Sensitization Pattern in Mite-Sensitized Rhinitis/ASTHMATIC Patients

Using hierarchical cluster analysis, 17 allergen components were classified into 5 different sensitization clusters. The first cluster included 5 components (Pen a 1, Per

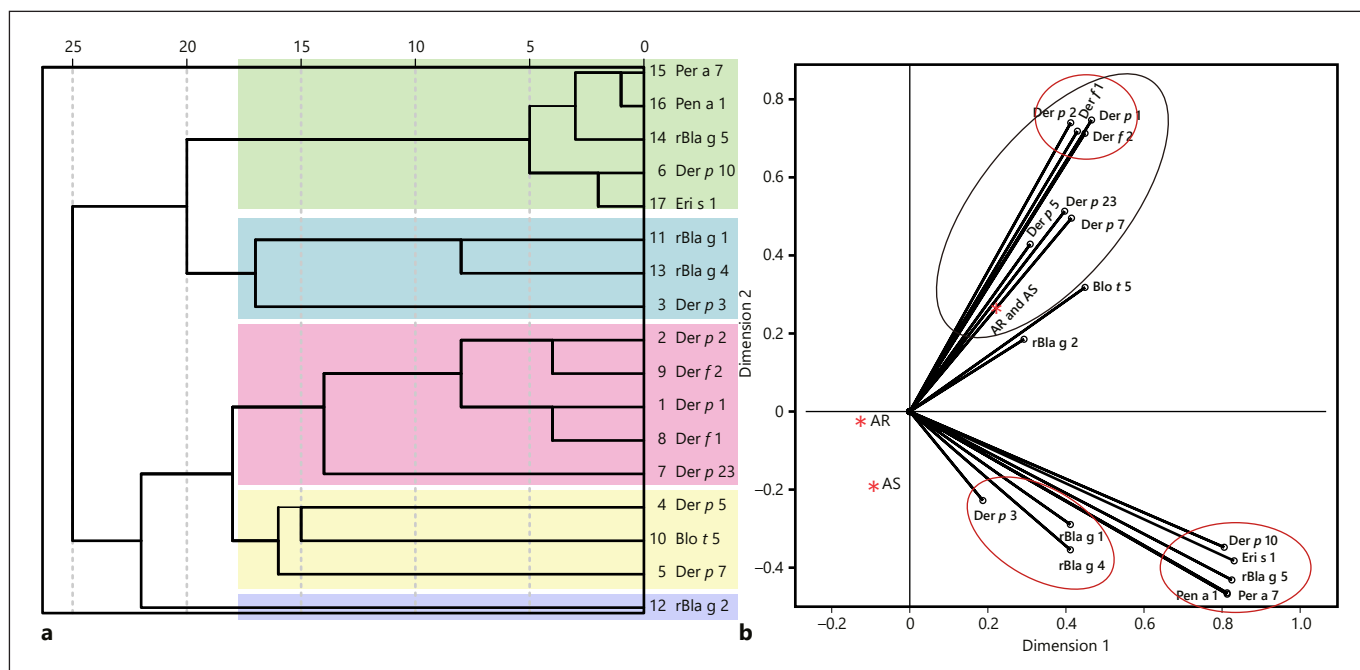


Fig. 2. Hierarchical cluster (a) and optimal scale (b) analysis of the connection between components. Hierarchical clustering analysis, also known as hierarchical cluster analysis, is an algorithm that groups similar objects into groups called clusters. The endpoint is a set of clusters, where each cluster is distinct from one another,

and the objects within each cluster are broadly similar to each other. Optimal scale analysis is a “dimensional reduction” analytical method. Using sIgE level and disease classification as the categorical variables, the closer the 2 points, the higher the correlation between the 2 factors. AR, allergic rhinitis; AS, asthma.

a 7, rBla g 5, Der p 10, and Eri s 1), the second cluster included 3 components (rBla g 1, rBla g 4, Der p 3), the third cluster included 5 components (Der f 1, Der p 1, Der f 2, Der p 2, Der p 23), and the fourth cluster included 3 components (Der p 5, Der p 7, and Blo t 5). Additionally, rBla g 2 was independently the fifth cluster (Fig. 2a).

According to optimal scale analysis, the correlation of allergen components showed that Der p 10, Pen a 1, rBla g 5, Per a 7, and Eri s 1 had strongly sensitivity correlation, and a positive correlation was also observed in Der p 3, rBla g 1 and rBla g 4; Der p 1, Der p 2, Der p 23, Der f 1, and Der f 2 (Fig. 2b, red circle). Furthermore, optimal scale analysis revealed the relationship between the disease group and the allergen components, showing that the AR and AS group was significantly associated with the major components of Der p and Der f (Der p 1, Der p 2, Der p 5, Der p 7, Der p 23 and Der f 1, Der f 2; Fig. 2b, black circle).

Further Analysis of Co-Sensitization after Hierarchical Cluster Analysis

After hierarchical cluster analysis, we roughly divided the 17 allergen components into 5 clusters (where rBla g 2 was used as a single cluster), and further analyzed the

combined sensitization of each cluster of allergen components by plotting the Venn diagram (Fig. 3). The Venn diagram in Figure 3a showed that 27.78% (15/54) of the samples were all positive to the 5 components (Pen a 1, Per a 7, rBla g 5, Der p 10, and Eri s 1), and 7.41% (4/54) of the samples were positive in 4 components. Additionally, 16.67% (9/54) of the samples were positive for Der p 10 alone and 12.96% (7/54) for Eri s 1. Among the 11 samples, only 1 sample (9%) was simultaneously sensitized to Der p 3, rBla g 1, rBla g 4, and 1 sample (9%) was sensitized to both Der p 3 and rBla g 4 (Fig. 3b). The third cluster consists of 5 allergen components including Der f 1, Der f 2, Der p 1, Der p 2, and Der p 23. A total of 190 (51.08%) samples were positive on the abovementioned 5 components, and 293 samples (78.76%) were sensitized to >4 components. Moreover, in 372 samples, 80.38% (299/372) were positive for Der p 23, while 97.65% (208/213) of the Der p 23-positive samples were positive for the other components (Fig. 3c). With Der p 5, Der p 7, and Blo t 5 as belonging in the fourth category, 35 (19.34%) samples were sensitized to the 3 components, and 97 (53.59%) samples were co-sensitized in >2 components (Fig. 3d).

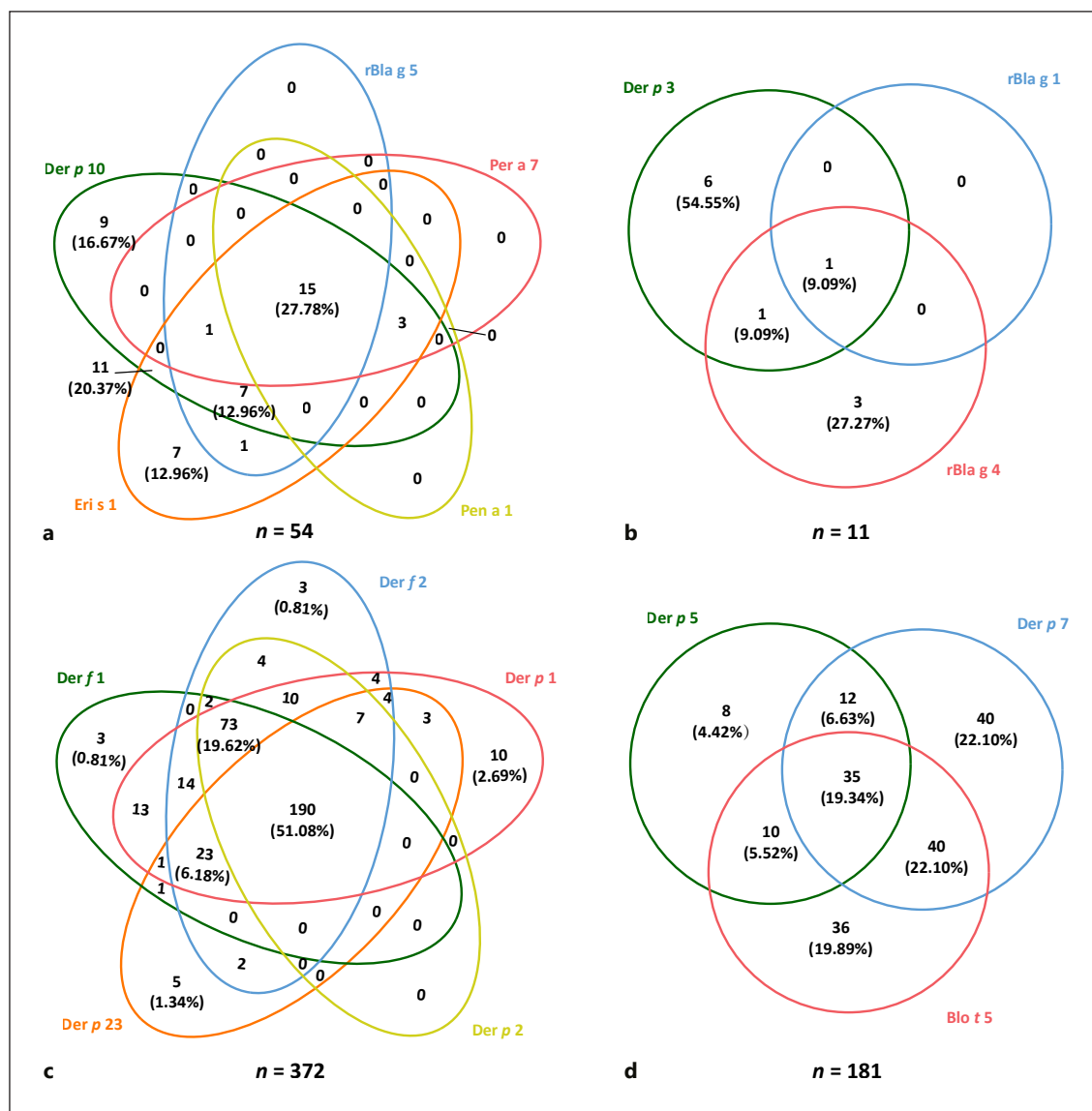


Fig. 3. a–d Venn diagram of component co-sensitization among the 4 clusters. Based on the hierarchical cluster analysis, the Venn diagram is plotted for each cluster. Moreover, the numbers inside the Venn diagram show the number of patients who were co-sensitized (positive) to >2 components.

CRD and Age

For most of the components, there was a significant difference in the sensitization rates among children, adolescents, and adults ($p < 0.05$; Fig. 4a), with the adult group having a significantly lower percentage than the children and adolescent groups, and the positive rate of the components in adolescents is slightly higher than the positive rate of children (the difference is not necessarily statistically significant). It can also be observed from Figure 4a that the sensitization rates of the components in

each cluster were similar. For example, the third clusters containing Der p 1, Der p 2, Der f 1, Der f 2, and Der p 23 components all had higher positive rates. The second clusters included rBla g 1, rBla g 4, and Der p 3 components, and there was no significant difference in the percentage of sensitization in the 3 age groups. The number of components sensitized per patient is shown in the line graph (Fig. 4b). A total of 88.84% (191/215) for the children group, 96.30% (52/54) for the adolescent group, and 69.3% (113/163) for the adult group were positive for >2

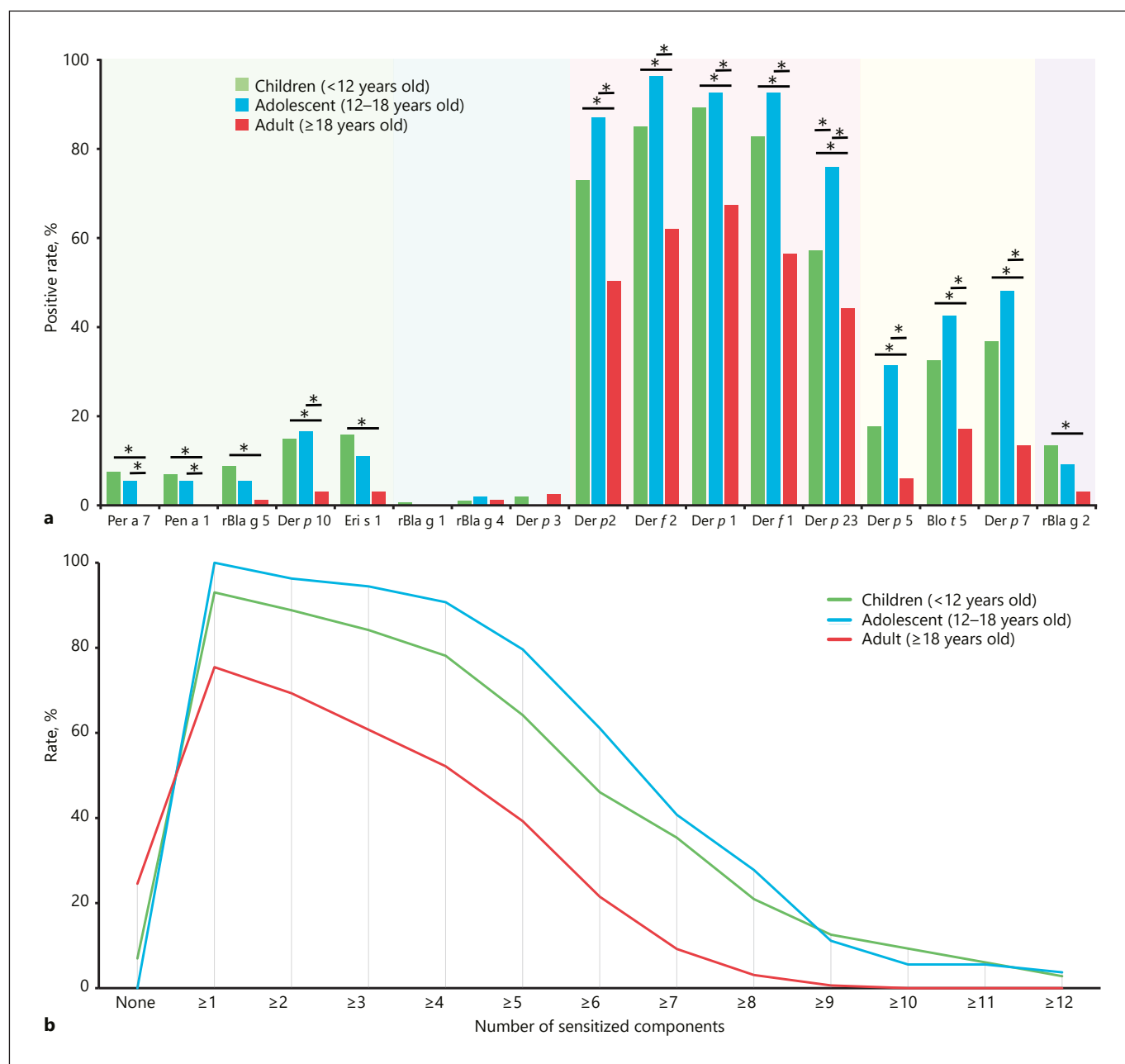


Fig. 4. Positive rate (percentage) of the 17 components in different age groups (**a**) and the percentage of patient's sensitization to the different numbers of components in RUOIMMUN. None indicates that none of the 17 tested components were positive, and 1–12 represents the number of positive individual components (**b**). Significant differences are shown: * $p < 0.05$, and different background colors are used to represent different cluster modes.

components. The percentage of poly-sensitive multiple components in the adult group was significantly lower than that in the adolescent and children groups, while adolescents were more likely to be sensitized to multiple components than children.

CRD and Total IgE

We also tested the total IgE (tIgE) concentration of the above 432 patients, and these patients were divided into the following 3 groups according to the tIgE concentration levels: low tIgE (<100 IU/mL), moderate tIgE (100–

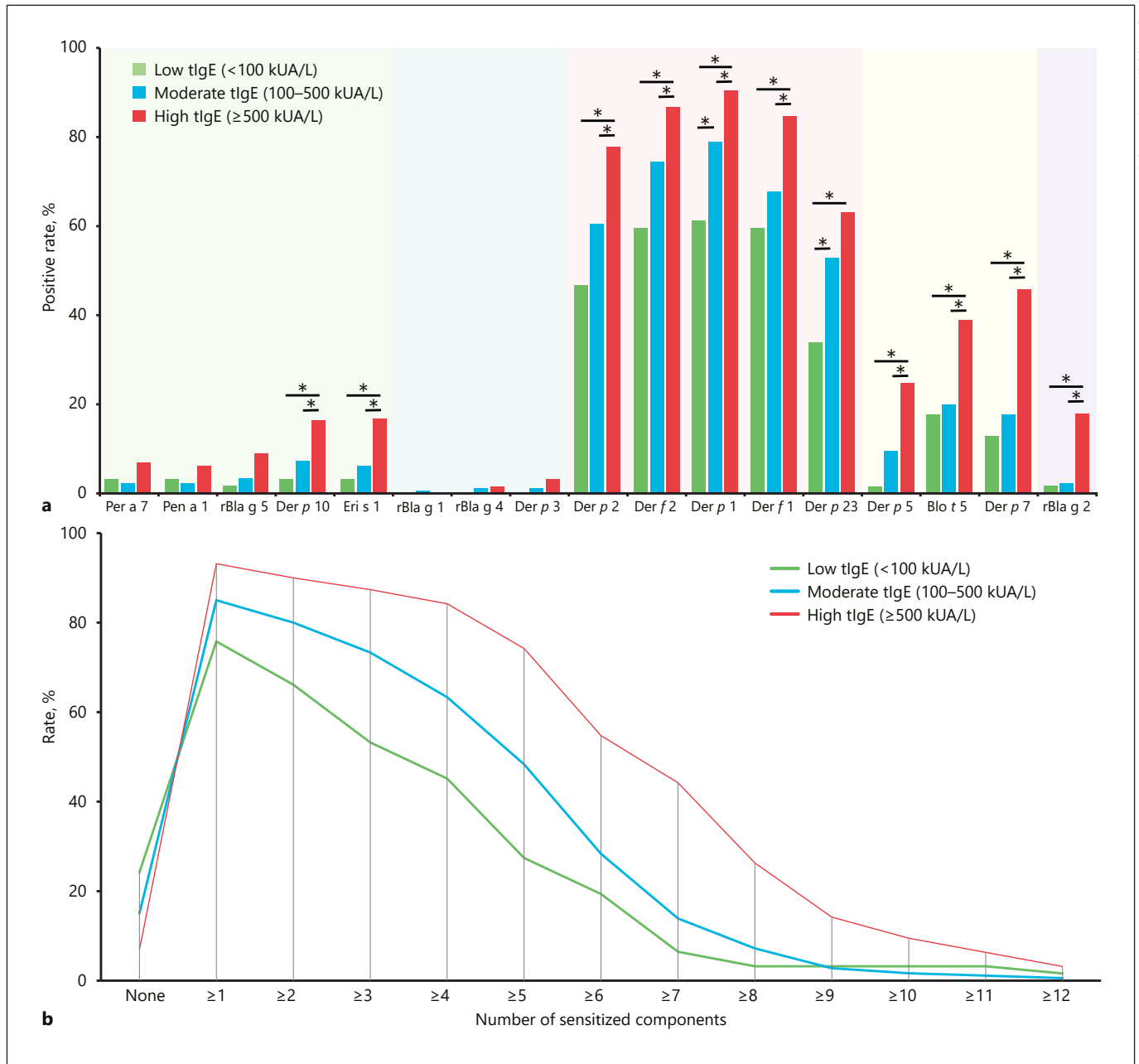


Fig. 5. Positive rate (percentage) of the 17 components in different total IgE level groups (a) and the percentage of patient's sensitization to the different numbers of components in RUROIMMUN. None indicates that none of the 17 tested components were positive, and 1–12 represents the number of positive individual components (b). Significant differences are shown: * $p < 0.05$, and different background colors are used to represent different cluster modes. tIgE, total IgE.

500 IU/mL), and high tIgE (≥ 500 IU/mL). Analysis of the difference in the positive rate of each component of the 3 tIgE groups (Fig. 5a) was performed. For the third cluster (Der p 2, Der f 2, Der p 1, Der f 1, Der p 23), fourth cluster (Der p 5, Blo t 5, Der p 7), and fifth cluster (rBla g 2), the

percentage of sensitization in the high tIgE group was significantly higher than that in the moderate and low tIgE groups. For Per a 7, Pen a 1, rBla g 5, Der p 10, and Eri s 1 components, the percentage of the high tIgE group is higher than that of the moderate and low tIgE groups.

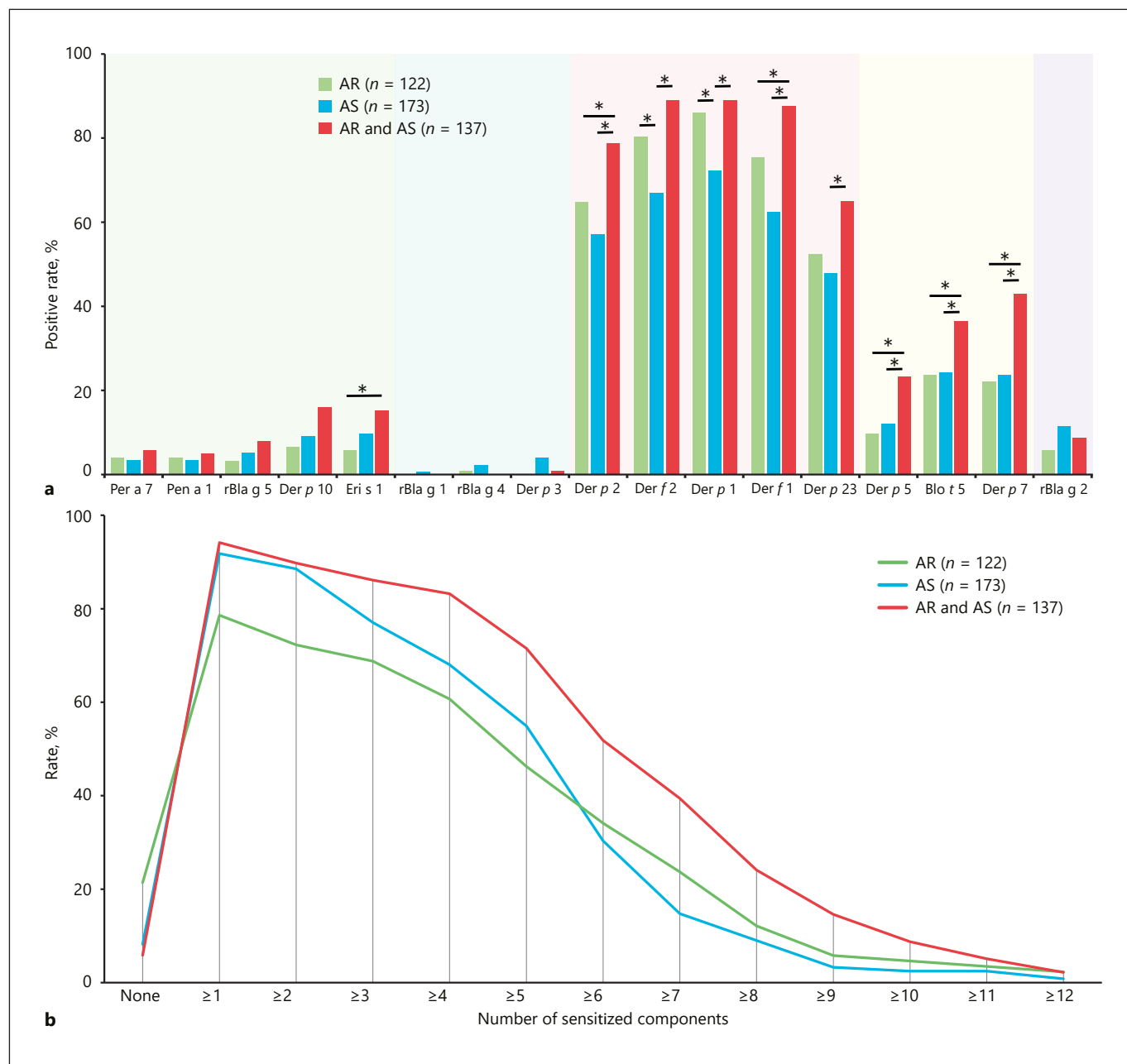


Fig. 6. Positive rate (percentage) of the 17 components in different disease groups (**a**) and the percentage of patient's sensitization to the different numbers of components in RUROIMMUN. None indicates that none of the 17 tested components were positive, and 1–12 represents the number of positive individual components (**b**). Significant differences are shown: * $p < 0.05$, and different background colors are used to represent different cluster modes. AR, allergic rhinitis; AS, asthma.

However, the difference was statistically significant only for Der p 10 and Eri s 1 components ($p < 0.05$). For rBla g 1, rBla g 4, and Der p 3 components, the sensitization percentage was not obviously different among the 3 tIgE level groups. A significant difference was observed be-

tween the low tIgE group and the moderate tIgE group only for Der f 1 and Der p 23 components ($p < 0.05$). For the high tIgE group, 90% of patients were positive for >2 components, while in the moderate tIgE group and the low tIgE group, 80% (144/180) and 66.13% (51/62) of pa-

tients were positive for >2 components, respectively. Moreover, it was easier to observe the phenomenon of combining multiple component sensitization in the high tIgE group, followed by patients with moderate tIgE levels (Fig. 5b).

CRD and Clinical Phenotypes

According to the different kinds of diseases, the patients were divided into the AR only, AS only, and AR + AS groups. The sensitization differences of the components among the 3 disease groups were analyzed, and a line graph of combined sensitization numbers was plotted. As seen in Figure 6a, the AR only, AS only, and AR + AS groups showed similar sensitization profiles in each cluster, and Der *f* 1, Der *f* 2, and Der *p* 1 components had the highest sensitized percentage. However, for the third cluster component (Der *p* 2, Der *f* 2, Der *p* 1, Der *f* 1, Der *p* 23), the positive rate was significantly higher in the AR + AS than group than in the AS only group. For Der *p* 2 and Der *f* 1, the percentages of the AR and AS group were significantly higher than that of the AR group, and for Der *f* 2 and Der *p* 1, the percentage of the AR group was higher than that of the AS group (all $p < 0.05$). In the fourth cluster component (Der *p* 5, Blo *t* 5, Der *p* 7), the sensitization percentage of the AR + AS group was significantly higher than that of the AR only and AS only groups. A total of 89.78% (123/137) of patients in the AR + AS group, 88.52% (108/122) in the AR only group, and 72.25% (125/173) in the AS only group were positive for >2 components. Moreover, patients with combined AR and AS were more likely sensitive to several components than those with AR or AS only. For example, 48.28% of AR + AS patients were sensitive to >5 components, in contrast to 30.47% in AR only and 32.50% in AS only patients (Fig. 6b).

Discussion

Previous studies have confirmed that house dust mites (HDM) including Der *p*, Der *f*, and Blo *t* are common allergens worldwide and are closely related to many allergic diseases, especially AR and (or) AS [14, 15]. It has also been confirmed from our previous study that mite and cockroach allergens are the most important inhaled allergens in respiratory allergic diseases in southern China and that shrimps and crabs are the most important food allergens [16]. In the current study, we selected mite-sensitized patients with respiratory allergies as the subjects and used CRD method to determine the co-sensitization

and cross-reactivity profiles of these major allergens at the molecular level.

This study tested the 7 components of Der *p*, including Der *p* 1, Der *p* 2, Der *p* 3, Der *p* 5, Der *p* 7, Der *p* 10, and Der *p* 23. Among them, Der *p* 1 (94.52%), Der *p* 2 (78.36%), and Der *p* 23 (63.29%) were the major components, which is consistent with the previous studies [6, 17]. Moreover, Der *f* 1 (86.7%) and Der *f* 2 (91.06%) had a high positive rate, indicating that Der *f* 1 and Der *f* 2 are the major allergens of Der *f*. Although the sensitization frequency of Blo *t* 5 in the overall population was 28.01%, it had a positive rate of 54.55% in Blo *t* sensitized subjects, which is similar to the Gonzalez-Perez et al. [18] study in patients with severe rhinitis caused by mite-sensitization. And Nge Cheong et al. [19] described 49% (22/45) sensitized to Blo *t* 5 in the Blo *t* positive population of respiratory allergic patients in China. We attempted to combine the major components of the 3 mites (including Der *p* 1, Der *p* 2, Der *f* 1, Der *f* 2, Blo *t* 5) for a comprehensive diagnosis and found that >80% of patients can be correctly diagnosed with mite allergy [20]. We screened Bla *g* positive samples and recalculated their component positive frequencies, rbla *g* 2 (15.56%) > rbla *g* 5 (8.89%) > rbla *g* 4 (4.44%) > rbla *g* 1 (1.11%). This is consistent with the findings of Pomés et al. [20]: there was no allergen dominating the response to cockroach (i.e., >50%).

In this study, a total of 17 components were detected, and we performed hierarchical cluster and optimal scale analysis to determine the potential relationship between molecules. The result of hierarchical cluster analysis shows that the 17 components can be roughly divided into 5 clusters, with Der *p* 10, Pen *a* 1, rBla *g* 5, Per *a* 7, and Eri *s* 1 classified as the first cluster. It is known that Der *p* 10, Pen *m* 1, and Per *a* 7 are all tropomyosin [21], rBla *g* 5 is a Glutathione-S-transferase [22], and Eri *s* 1 is one of the components present in crabs. According to our results, >1/4 of the samples were both sensitized to the above 5 components, and 70.37% were sensitized to >2 components (Fig. 3a). Moreover, the results of the scale analysis also showed that there was a strong correlation and similarity among the 5 components (Fig. 2b, red cycle). Several studies have confirmed cross-reactivity between mites, cockroaches, shrimps, and crabs, which are associated with tropomyosin [23, 24]. In this study, the positive rate of Der *p* 10 in patients with mite-sensitized respiratory allergies was 10.65%, which was highly consistent with the study results of Sastre-Ibanez [4], and the percentage was higher than that previously reported in China [25, 26].

The components of the second cluster include Bla g 1, rBla g 4, and Der p 3, with rBla g 1 (nitrile specifier) and rBla g 4 (lipocalin) being the components of German cockroach [27] and Der p 3 (trypsin) is one of Der p component. The 3 components had the lowest positive rate of sensitization in the cohort of this study (<1.85%). Trypsin from Der p has been shown to be a single-chain protein with similar properties to other insects' trypsin [28]. However, studies emphasizing the co-sensitizing phenomenon between the 3 components were insufficient. We believe that the 3 components were classified into one cluster because of their very low positive rate in this study. Furthermore, the result of the Venn diagram, that is, that the co-sensitizing rate of the 3 components was only 9%, supported our conclusion (Fig. 3b), but this needs to be verified by conducting more experiments.

The third cluster group included Der p 1, Der p 2, Der p 23, Der f 1, and Der f 2. These 5 components were the major sensitizing components in this study cohort, and all of them had the highest positive rate of sensitization (all >50%). Der p 23 is a protein of the peritrophic matrix that lines the midgut and surrounds the digested food stuff and the fecal pellets, whose epidemiological level is another important HDM allergen other than Der p 1 and Der p 2 [29], which means it truly sensitized the HDM [30]. These 5 components are classified into the same category because they have a high rate of common sensitization in mite-sensitized patients and have a strong correlation (Fig. 2b, red cycle). From our results, we also observed that a large proportion of patients with positive sensitization rate in this cluster had a high level (class 4–5) of sIgE concentrations, a phenomenon that was not described in other related studies. A total of 82.41% of the population in this study showed multicomponent sensitization, which may mean a higher level of sIgE concentration. The fourth cluster includes Der p 5, Der p 7, and Blo t. Blo t 5 is the major allergen of Blo t, and serological epidemiological data show that it has partial cross-reactivity with allergens from Der p [31, 32]. Caraballo et al. [32] performed the radioallergosorbent test and Western blot inhibition assay to directly assess the presence of a cross-reaction between the recombinant component rDer p 5 of Der p and Blo t 5 of Blo t [33], but they did not further report the association between the presence of cross-reaction and Der p 7. rBla g 2, a gut inactive aspartic protease, was one of the most important allergens from cockroach and means true sensitivity to cockroaches instead of a cross sensitive component [27, 34]. For the overall population in this study, the sensitive frequency of crude extract Bla g was 20.83% (90/432),

while that of rBla g 2 was 9.03%. These results suggested that rBla g 2 was not the main sensitizing component in this population, so it may not be strongly co-sensitive to other components. Therefore, when the hierarchical cluster analysis was run, rBla g 2 was classified as a separate cluster.

Regardless of age, tIgE levels, and disease type factors, similar sensitization profiles were observed for each component in the same category based on hierarchical clustering analysis. For example, in children and adolescent groups, high tIgE group (≥ 500 IU/mL), and the AR + AS group, all components of the third cluster, including Der p 2, Der f 2, Der p 1, Der f 1, and Der p 23, had higher sensitizing rates. Aging can significantly affect the function of the immune system [35], and several studies have also shown that the prevalence of allergies decreases with age, while IgE level also declines [36, 37]. Consistent with the other studies, most of the examined allergen components had significantly lower positive rates of sIgE in the adult group than that in the children and adolescent groups (all $p < 0.05$), but there was no difference in the second cluster components (rBla g 1, rBla g 4, Der p 3) among the different age groups. The results of multiple sensitizing percentages (Fig. 4b) in different age groups showed that adolescent patients were likely to be sensitized with multiple components simultaneously, followed by children. Many epidemiological studies have shown that there is a strong relationship between the responsiveness of serum tIgE concentration to aeroallergens and the prevalence of AS [38]. For the major allergen components, high serum tIgE level had a higher positive rate of allergen, and more components were sensitized together. Patients in the 3 disease groups had similar allergen distribution to most of the allergen components, suggesting that AR + AS are very similar in etiology, immunology, pathogenesis, and allergen distribution [39, 40]. The poly-sensitization rates of patients with AR, AS, AR + AS were 77.25, 88.52, and 89.78%, respectively. Furthermore, patients with AR + AS are more likely to be sensitized to multiple components than patients with AR or AS alone, suggesting that most patients with AR + AS can simultaneously sensitize multiple components; hence, multiple allergens co-sensitization may increase the severity of the disease.

This study included a large number of patient samples and tried to identify potential co-sensitization and cross-reaction characteristics between allergen component proteins through CRD. However, there are some limitations in this study. The allergen sensitization characteristics are not related to the severity of the patient's condi-

tion, and the information such as the patient's symptom score was not obtained. In addition, since there is only one component of Blo *t* 5 commercially available, so we detected only one component of Blo *t* (one of the most important components Blo *t* 5), follow-up studies will introduce more allergen components to be linked to the patient's clinical symptoms.

Conclusion

Epidemiological data on allergen components causing allergic symptoms can be further understood using CRD. Der *p* 1, Der *p* 2, Der *p* 23, Der *f* 1, Der *f* 2 as the primary sensitizing component of the study cohort. The positive rate for Blo *t* 5 was 28.01% for all populations and 54.45% for Blo *t*-positive samples. In addition, CRD allows us to identify more potential allergen associations such as common sensitivities and cross-reactions between component proteins. Based on these results, we suggest that when patients are identified as sensitized to a particular allergen, Clinicians can pay more attention to other allergen components that are closely related to it.

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Statement of Ethics

This study and the use of the human serum samples were approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYYY-2016-73).

Disclosure Statement

The authors declare no financial conflicts of interest with this work.

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Author Contributions

B.S., Z.H., and W.L. conceived and designed the study, obtained approval. X.Z., Z.H., and C.L. collected clinical information and serum samples from clinical patients. X.Z., Z.H., and H.C. performed the detection of allergen-specific IgE for all samples. B.S., Z.H., W.L., and H.H. analyzed the data. B.S., Z.H., X.Z., and H.C. drafted the manuscript in close collaboration with all co-authors. All authors read and approved the final version.

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