

Received: 19 April 2018

Revised: 20 July 2018

Accepted: 31 August 2018

DOI: 10.1111/all.13597

## ORIGINAL ARTICLE

WILEY  Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

## Airway Diseases

# Artemisia pollen allergy in China: Component-resolved diagnosis reveals allergic asthma patients have significant multiple allergen sensitization

Zhongshan Gao<sup>1,2,3</sup>  | Wan-Yi Fu<sup>2</sup> | Yuemei Sun<sup>4</sup> | Biyuan Gao<sup>5</sup> | Hui-Ying Wang<sup>6</sup> | Meiling Liu<sup>7</sup> | Fang-Mei Luo<sup>8</sup> | Xiang Zhou<sup>2</sup> | Jing Jin<sup>2</sup> | Lan Zhao<sup>2</sup> | Shandong Wu<sup>1</sup> | Yi Liu<sup>1</sup> | Lingying Wu<sup>7</sup> | Xuefeng Wang<sup>7</sup> | Ning-Bo Tang<sup>4</sup> | Bao-Hua Guo<sup>8</sup> | Yan Feng<sup>9</sup> | Jian Ying Zhou<sup>10</sup> | Gabriele Gadermaier<sup>11</sup>  | Fatima Ferreira<sup>11</sup>  | Serge A. Versteeg<sup>3</sup> | Ronald van Ree<sup>3</sup>

<sup>1</sup>Allergy Research Center, Zhejiang University, Hangzhou, China<sup>2</sup>College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China<sup>3</sup>Department of Experimental Immunology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands<sup>4</sup>Department of Allergy, Yu Huang Ding Hospital, Yantai, China<sup>5</sup>Hangzhou Aileji Biotech Ltd, Hangzhou, China<sup>6</sup>Department of Allergy, The Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China<sup>7</sup>Department of Allergy, The Third People's Hospital of Datong, Shanxi, China<sup>8</sup>Department of Otorhinolaryngology, Qujing Chinese Traditional Medicine Hospital, Yunnan, China<sup>9</sup>Department of Otorhinolaryngology, The First Affiliated Hospital, Shanxi Medical University, Taiyuan, China<sup>10</sup>Department of Respiratory Disease, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China<sup>11</sup>Department of Biosciences, University of Salzburg, Salzburg, Austria**Correspondence**

Zhongshan Gao, Allergy Research Center and College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China.  
Email: gaozhongshan@zju.edu.cn

**Funding information**

Hangzhou Aileji Biotech Ltd, China, Grant/Award Number: 2016-R1; National Natural Science Foundation of China, Grant/Award Number: 31772271

**Abstract**

**Background:** *Artemisia* pollen allergy is a major cause of asthma in Northern China. Possible associations between IgE responses to *Artemisia* allergen components and clinical phenotypes have not yet been evaluated. This study was to establish sensitization patterns of four *Artemisia* allergens and possible associations with demographic characteristics and clinical phenotypes in three areas of China.

**Methods:** Two hundred and forty patients allergic to *Artemisia* pollen were examined, 178 from Shanxi and 30 from Shandong Provinces in Northern China, and 32 from Yunnan Province in Southwestern China. Allergic asthma, rhinitis, conjunctivitis, and eczema symptoms were diagnosed. All patients' sera were tested by ImmunoCAP with mugwort pollen extract and the natural components nArt v 1, nArt ar 2, nArt v 3, and nArt an 7.

**Abbreviations:** CI, 95% confidence interval; CRD, component-resolved diagnostics; OR, odds ratio; SD, Shandong province, Northern China; SX, Shanxi province, Northern China; YN, Yunnan province, Southwestern China.

Gao, Fu and Sun contributed equally to this research.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2018 The Authors. *Allergy* Published by John Wiley & Sons Ltd.

**Results:** The frequency of sensitization and the IgE levels of the four components in *Artemisia* allergic patients from Southwestern China were significantly lower than in those from the North. Art v 1 and Art an 7 were the most frequently recognized allergens (84% and 87%, respectively), followed by Art v 3 (66%) and Art ar 2 (48%). Patients from Northern China were more likely to have allergic asthma (50%) than patients from Southwestern China (3%), and being sensitized to more than two allergens increased the risk of allergic asthma, in which co-sensitization to three major allergens Art v 1, Art v 3, and Art an 7 is prominent.

**Conclusions:** Component-resolved diagnosis of Chinese *Artemisia* pollen-allergic patients helps assess the potential risk of mugwort-associated allergic asthma.

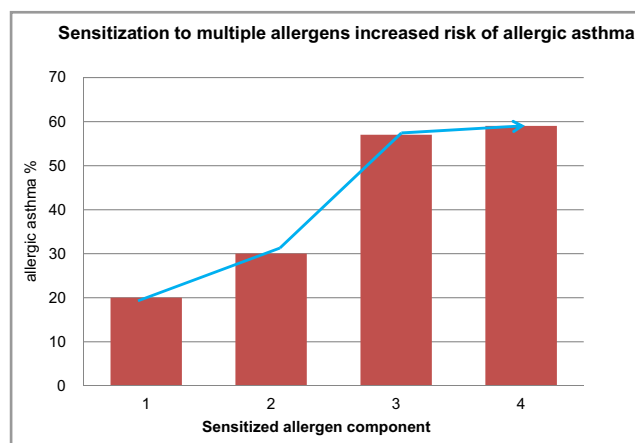
#### KEYWORDS

*Artemisia* pollen allergy, asthma, component-resolved diagnosis

## 1 | INTRODUCTION

Over the past 30 years, there has been a significant increase in the prevalence of allergy in the Chinese population, and allergy has been found to be the major factor causing asthma.<sup>1,2</sup> A cross-sectional study has shown that overall, while about 11.3% of patients in China with respiratory allergies are sensitized to *Artemisia* pollen, this value is much higher in Northern China (>50%). Among the major pollen species causing seasonal allergic asthma in China, *Artemisia* pollen has the highest association index.<sup>3-7</sup> The hop (*Humulus scandens*), ragweed (*Ambrosia artemisiifolia*), and goosefoot (*Chenopodium album*) pollen seasons overlap with that of *Artemisia*, but their IgE positive rate was significantly lower in a survey of a large number of ImmunoCAP tests in Beijing.<sup>5</sup> In some areas in Europe, mugwort (*A. vulgaris*) pollen has also been associated with late summer and autumn hay fever and asthma, but its clinical importance is limited compared to birch and grass pollen.<sup>8</sup>

Component-resolved diagnosis (CRD) enables IgE sensitization profiles and potential risk to be established, and whether specific IgE is the result of primary sensitization or cross-reactivity can also be determined.<sup>9-13</sup> Such information can assist in making the right choice of immunotherapy, particularly when pollen seasons of different allergenic sources overlap.<sup>14-19</sup> Seven *Artemisia* pollen allergens have been identified and listed in the IUIS database ([www.allergen.org](http://www.allergen.org)), including the recently added 62 kDa putative galactose oxidase protein (Art an 7), first purified from sweet wormwood (*A. annua*).<sup>20,21</sup> In earlier studies with nine patients in Europe, using array-based profiling of five mugwort allergens, Art v 1, Art v 3, Art v 4, and Art v 6 were recognized by 60%-80% patients, with a co-sensitization frequency of 60% of three of these four allergens.<sup>22</sup> Until now, only two mugwort major component allergens (Art v 1 and Art v 3) have been commercially available for component-resolved diagnosis, with the prevalence of Art v 1 around 80% and Art v 3 around 60% in mugwort-allergic patients from Beijing and Shanxi provinces.<sup>21,23</sup> Previous CRD analyses have revealed Art v 1 as a specific



#### GRAPHICAL ABSTRACT

Component-resolved diagnosis of Chinese *Artemisia* pollen allergic patients helps assess the potential risk of mugwort-associated allergic asthma. Sensitization to more than two *Artemisia* pollen allergens resulted in higher risk of allergic asthma, in which co-sensitization to Art v 1, Art v 3 and Art an 7 allergens is prominent.

marker of mugwort pollen sensitization, and it is on the list of the 100 important allergen molecules,<sup>19</sup> although significant differences were found in different geographic areas.<sup>24</sup>

Currently, conventional diagnosis of *Artemisia* pollen allergy in China largely relies on skin prick tests, immunoblot kits using European mugwort or Chinese *Artemisia* spp. pollen extracts, or ImmunoCAP (*w6*, *A. vulgaris* extract); CRD has rarely been applied to establish sensitization profiles or to direct allergen immunotherapy in Chinese mugwort-allergic patients. In addition, the relation between IgE responses to different components and clinical phenotypes has barely been explored.<sup>7</sup> Here, we used ImmunoCAP to evaluate the importance of IgE to four *Artemisia* pollen components: Art v 1 (defensin-like protein), Art ar 2 (pathogenesis-related protein 1), Art v 3 (lipid transfer protein), and Art an 7 (putative galactose oxidase) in a group of 240 Chinese *Artemisia* pollen-allergic patients from three

different Chinese provinces (Shanxi, Shandong, and Yunnan, respectively) with characteristic seasonal *Artemisia* pollen counts, and describe possible associations with demographics and clinical phenotypes.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients and symptoms

A total of 240 patients were recruited based on a convincing history of *Artemisia* pollen allergy in late summer and autumn, positive results in specific mugwort IgE tests but negative or very weak to hop pollen (w22) by ImmunoCAPs or by quick immunoblot kit assay (EUROIMMUN Beijing, China or Xinhualian, Beijing, China). These 240 patients were from three areas: 178 from Datong and Taiyuan, Shanxi Province (SX), 30 from Yantai, Shandong Province (SD), and 32 from Qujing, Yunnan Province (YN). CRD for some of the patients from SX (110/178) has previously been reported,<sup>21</sup> so for these, here, only the Art ar 2 component was tested. Patients were also divided into three age groups—children (2-12 years old), teenagers (13-18 years), and adults (19-66 years). The three areas in China showed different seasonal *Artemisia* pollen counts: Taiyuan (Shanxi province), inland next to the Inner Mongolia grasslands, has the highest *Artemisia* pollen concentration which reaches a peak in August and September; in the coastal city of Yantai (Shandong province), east of Shanxi, the pollen peak is in September; and the Yunnan province, with a subtropical and tropical monsoon climate, has a very low *Artemisia* pollen concentration, limited to October and November. Geographic areas and *Artemisia* pollen count records (microscopic observation of pollen collected on a slide by a simple device) were taken from a survey report<sup>25</sup> and are given in Figure S1.

Clinical presentations of *Artemisia* pollen allergy were established by experienced clinicians through questionnaires and clinical observations and tests. Seasonal allergic asthma was defined by a history of dyspnea, wheezing and/or coughing episodes and diagnosed by spirometry (MasterScreen or Powercube-Body, Germany). For some patients (33/104) from SX and SD, asthma was confirmed by testing for positive airway reversibility, that is, by FEV<sub>1</sub> increasing  $\geq 12\%$  after inhalation of 400  $\mu\text{g}$  of salbutamol sulphate. Conjunctivitis was defined based on symptoms such as ocular itching and tearing. The diagnosis of atopic eczema was based on the presence of skin rash with red, raised itchy bumps, and of allergic rhinitis on itching, sneezing, runny nose, and congestion. For all clinical presentations, a clear association of symptoms with the *Artemisia* pollen season was necessary for a diagnosis of *Artemisia* pollen allergy.

Written informed consent was obtained from all participants, and the study was approved by the local ethics committee of Second Affiliated Hospital, College of Medicine, Zhejiang University (authorization No. 2011-R-1 and 2013-67-087) in collaboration with the Third People's Hospital of Datong, Shanxi (authorization No. 2015-001), Yu Huang Ding Hospital, Yantai, Shandong (authorization No. 2015-164), and Qujing Chinese Traditional Medicine Hospital, Yunnan, China.

### Highlights

- The frequency and IgE levels of four *Artemisia* allergen components in patients from SX and SD were higher than that in YN China.
- Patients from SX and SD were more likely to have allergic asthma than patients from YN China.
- Patients co-sensitization to Art v 1, Art v 3 and Art an 7 have a higher risk of developing allergic asthma.

### 2.2 | ImmunoCAP tests

All 240 subjects were tested for their specific IgE to mugwort pollen extract (code w6) and two commercial components (nArt v 1, nArt v 3, with code w231 and w233, respectively) by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden). The two additional natural allergens Art ar 2 (GenBank MF326217) and Art an 7 (GenBank KY428925) recently included in IUIS (<http://allergen.org/>) were biotinylated and coupled to streptavidin-conjugated ImmunoCAPs (o212) as described previously.<sup>26</sup> Natural Art ar 2 was purified by a monoclonal antibody affinity chromatography (C9-C1: obtained by immunization from a pollen extract of Chinese mugwort *A. argyi*), and its primary sequence is identical to Art v 2 as demonstrated upon cloning (GenBank MF326222). Natural Art an 7 was purified from *A. annua* extract as described previously.<sup>21</sup> Sera with an IgE value  $> 100 \text{ kU}_A/\text{L}$  were diluted and retested, with 0.35  $\text{kU}_A/\text{L}$  considered as positive cutoff.

### 2.3 | Statistical analyses

Data were analyzed by SPSS 21.0, with a value of  $P < 0.05$  considered as significantly different. Frequencies of positive IgE were analyzed by chi-square test or Fisher's exact test and differences in IgE levels between groups calculated by the Mann-Whitney  $U$  test. The Spearman correlation coefficient analysis was used to determine correlations of IgE levels between groups, and quantitative variables in the three areas were compared by Kruskal-Wallis test, with Bonferroni correction applied in pair-wise comparison.

## 3 | RESULTS

### 3.1 | Symptoms and sensitization profiles of Chinese mugwort-allergic patients

Of the 240 patients, the vast majority (95%) were diagnosed with *Artemisia* pollen-associated allergic rhinitis. In addition, 53% had conjunctivitis, 43% allergic asthma, and 19% atopic eczema, with quite significant geographic variation of these three symptoms (Table 1). For IgE reactivity against the four components, Art v 1 was the most frequently recognized allergen (81%, range 53%-93%), ahead of Art an 7 (80%, range 38%-87%), Art v 3 (53%, range 9%-66%) and Art v

**TABLE 1** Demographics and characterization of the study population in the three provinces in China

	SX	SD	YN	Total	P value
Patients, no.	178	30	32	240	
Gender, female no. (%)	92 (52)	18 (60)	18 (56)	128 (53)	0.653
Age, median (range)	22 (4-61)	23.5 (3-66)	31.5 (6-54)	23 (3-66)	0.453
Symptoms (%)					
Asthma	85 (48)	18 (60)	1 (3)	104 (43)	<0.001
Conjunctivitis	92 (52)	7 (23)	27 (84)	126 (53)	<0.001
Rhinitis	166 (93)	29 (97)	32 (100)	227 (95)	0.324
Eczema	36 (20)	2 (7)	7 (22)	45 (19)	0.201
Specific IgE, median, IQR (kU <sub>A</sub> /L)					
Mugwort extract	39.3 (16.3-78.4)	25.2 (7.5-74.7)	2.8 (1.2-11.9)	30.4 (10.5-70.8)	<0.001
Art v 1	11.7 (1.8-30.3)	13.7 (4.5-64.5)	0.4 (0.0-5.5)	9.6 (1.1-29.0)	<0.001
Art ar 2	0.3 (0.1-1.3)	0.3 (0.1-0.9)	0.0 (0.0-0.1)	0.2 (0.1-1.0)	<0.001
Art v 3	15.8 (0.1-56.7)	0.1 (0.0-0.7)	0.0 (0.0-0.0)	1.1 (0.1-44.4)	<0.001
Art an 7	3.1 (1.0-8.5)	0.9 (0.5-3.1)	0.1 (0.0-0.6)	2.1 (0.5-6.3)	<0.001

SD, Shandong province; SX, Shanxi province; YN, Yunnan province.

2 (42%, range 9%-48%). Median IgE levels were highest against Art v 1 (9.6 kU<sub>A</sub>/L), followed by Art an 7 (2.1 kU<sub>A</sub>/L) and Art v 3 (1.1 kU<sub>A</sub>/L), whereas the median IgE to Art ar 2 was below the cutoff (Table 1).

The correlation of mugwort IgE levels to the sum of the four components was very high (Spearman  $r = 0.92$ ,  $P < .001$ ), fitting a linear regression ( $R^2 = 0.74$ , Figure S2). The 11 outliers in the bottom right corner are mainly from YN Province. For these patients, other allergens such as Art v 4 and/or Art v 6 may play a role.

### 3.2 | CRD and age

IgE levels against mugwort extract, Art ar 2, and Art an 7 were significantly different ( $P < 0.05$ ) among children, teenagers and adults (Figure 1A), with the adult group having a significantly lower IgE to mugwort and Art an 7 than the child and teenager group. The frequency of sensitization to Art ar 2 was higher in the child group than in the teenager and adult groups (Figure 1B). The number of components recognized per patient was not significantly different between age groups (Figure 1C).

### 3.3 | CRD and geographic background

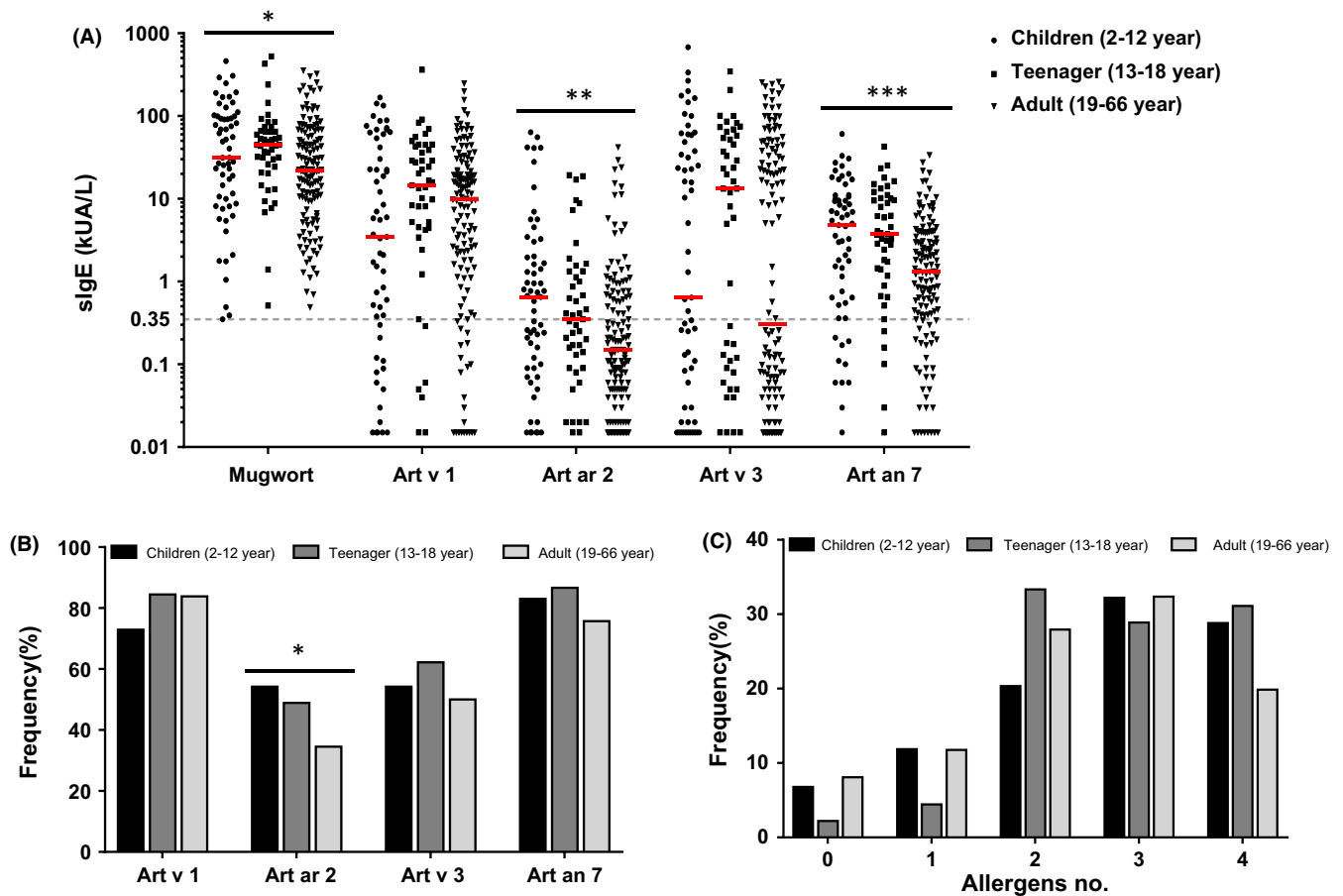
The IgE levels against mugwort extract and four allergen components in Shanxi (SX) and Shandong (SD) in Northern China were significantly higher than in patients from Yunnan (YN) in Southwestern China ( $P \leq 0.001$ ). SX patients had higher IgE levels against the two components Art v 3 and Art an 7 than patients from SD, but not against Art v 1 and Art ar 2 (Figure 2A). The frequency of sensitization to Art v 1, Art ar 2, and Art an 7 was similar in SX and SD, and significantly higher than in YN. The number of SX patients sensitized to Art v 3 was significantly higher and with higher IgE level (Figure 2A,B). Most patients

from SX and SD were sensitized to 2-4 components, while for YN patients this was less than 2 components (Figure 2C). Multiple allergen sensitization (3-4 components) resulted in an elevated occurrence of allergic asthma ( $P < 0.001$ , OR, 3.65; 95%CI, 2.11-6.33). Patients from SX and SD in N China were more likely to have allergic asthma (48%-64%) than patients from YN (3%, in SW China). Rates of conjunctivitis were very high in YN patients, followed by SX patients, with the lowest rate found in SD ( $P < 0.01$ ) (Figure 2D).

### 3.4 | CRD and clinical phenotypes

For all tested patients, mugwort extract IgE values in the allergic asthma group were higher than the non asthmatic group but not significantly, while three components Art v 1, Art v 3, and Art an 7 showed a statistically significant difference (Figure 3A). With increasing numbers of allergens recognized, the frequency of allergic asthma increased, but this was not observed for the other three allergic symptoms (Figure 3B). Furthermore, the frequency of sensitization to Art v 1, Art v 3, and Art an 7 was also significantly higher in patients with allergic asthma (Figure 3C), resulting in an approximately 3-fold higher risk of allergic asthma in patients sensitized to Art v 1 (OR, 3.26; 95% CI, 1.53-6.94;  $P = 0.002$ ), Art v 3 (OR, 2.77; 95% CI, 1.63-4.72;  $P = 0.001$ ), and Art an 7 (OR, 3.28; 95% CI, 1.58-6.78;  $P = 0.001$ ).

To exclude the influence of geographic background on association of sensitization profiles and age with allergic asthma, analyses were performed for the 178 patients from SX alone. There were no significant differences between asthmatic and non asthmatic groups for IgE values of mugwort extracts and four individual components (Figure 4A). There was a weak but significant correlation between patients' age and asthma symptoms (Spearman  $r = 0.284$ ,  $P < 0.001$ ), with adults suffering more often from asthma than children (Table 2, Figure 4B). Asthmatic patients had a higher frequency of IgE reactivity to three or four allergens than the non asthmatic patients (0-2



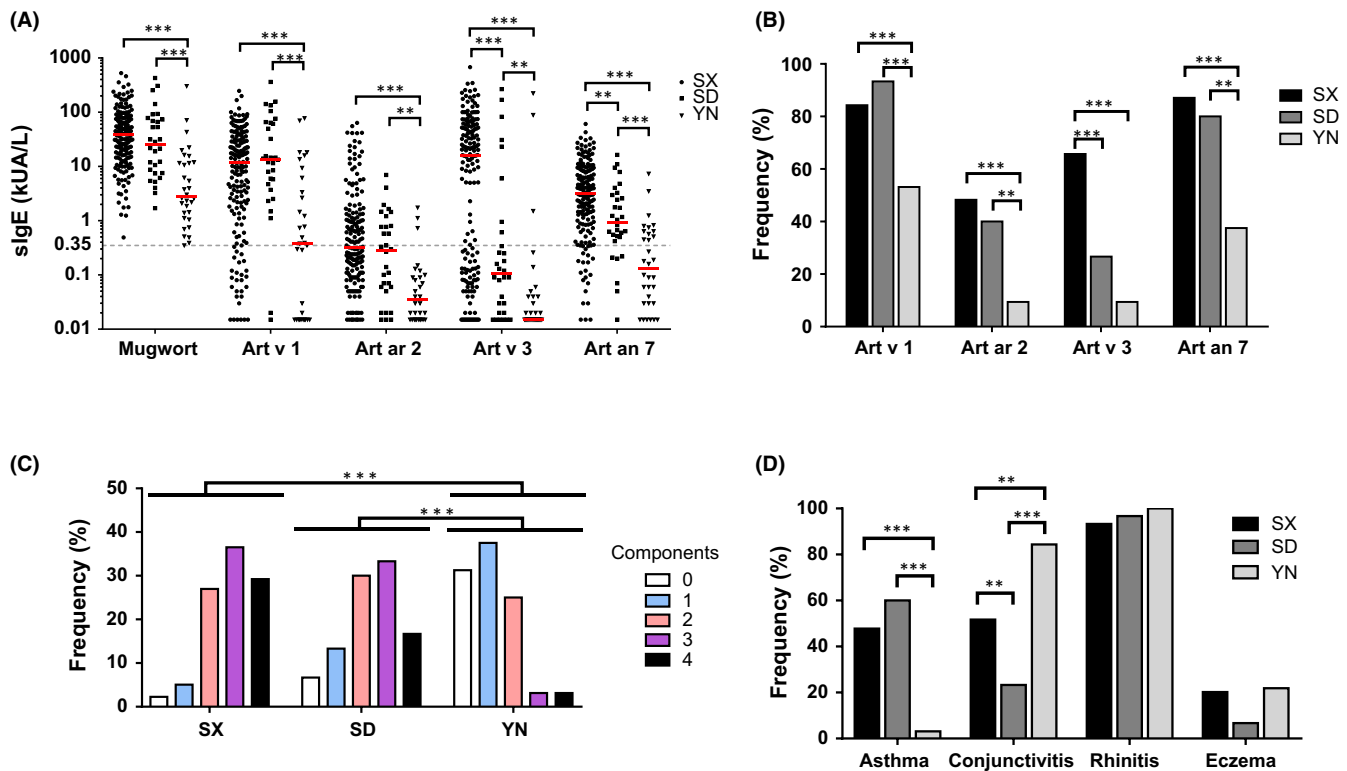
**FIGURE 1** Mugwort extract and four components tested in different age groups. IgE levels (A), frequency (B) and number of sensitized allergens, 0 indicates none of the four tested components were positive, and 1, 2, 3, and 4 represent the number of positive individual allergens Art v 1, Art ar 2, Art v 3, and Art an 7 (C). Significant differences are shown: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

components) ( $P = 0.001$ , OR, 2.86, 95% CI, 1.49-5.50) (Figure 4C). The sensitization frequency to Art v 1 ( $P = 0.027$ ) and Art v 3 ( $P = 0.01$ ) was significantly higher than in the non asthma group (Table 2), and more than a 2-fold risk of allergic asthma was found in *Artemisia* pollen-allergic patients with IgE reactivity to Art v 1 (OR, 2.64; 95% CI, 1.09-6.36) and Art v 3 (OR, 2.30; 95% CI, 1.21-4.37). No significant differences were observed for the other two allergens (Table 2). The interrelation of symptoms and sensitized allergens in asthmatic and non asthmatic groups is shown in Figure 4D,E, with the number of patients reacting to both Art v 1 and Art v 3 being significantly higher ( $P < 0.03$ ) in asthmatic (72.6%) than non-asthmatic allergy patients (48.9%).

## 4 | DISCUSSION

This study provides new insights in mugwort component sensitization and clinical significance in China. From both sensitization frequencies and IgE titers, we can draw a general conclusion that three allergens qualify as major allergens in China: Art v 1, Art v 3, and Art an 7. These results are in line with those from Europe<sup>27</sup> and our

immunoblots of three widely recognized allergens in Northern China.<sup>21</sup> Art v 1 and Art an 7 were consistently the most prevalent (more than 80%) in the three areas, indicating that these two allergens are biomarkers of *Artemisia* pollens inducing allergic reactions. From the observations obtained from Shanxi and Shandong, we estimate the prevalence of Art v 1 at 84%-93% in Northern China, which is quite similar to that of the mugwort-allergic patients in Europe but higher than in North America.<sup>24,27</sup> Median Art v 1 IgE value was also similar to those in Northern Europe. Art an 7 was assumed to be in the same allergen group as Art v 60 kDa, which was reported about 20 years ago and has recently been identified as a putative galactose oxidase with a very high rate of sensitization (around 90%).<sup>21</sup> In the present study, the frequency of Art an 7 sensitization was quite similar, at 87% in SX and slightly lower in SD. The prevalence and degree of sensitization to Art v 3 were quite different in these two areas, with 66% in SX and 25% in SD. Very significant differences with respect to recognition of Art v 3 amongst mugwort-allergic patients have also been reported in Europe.<sup>20</sup> Although the prevalence of 66% in SX is quite similar to that reported in central Europe,<sup>10,28</sup> IgE levels are much higher. The striking difference between SX and SD is most likely explained by a



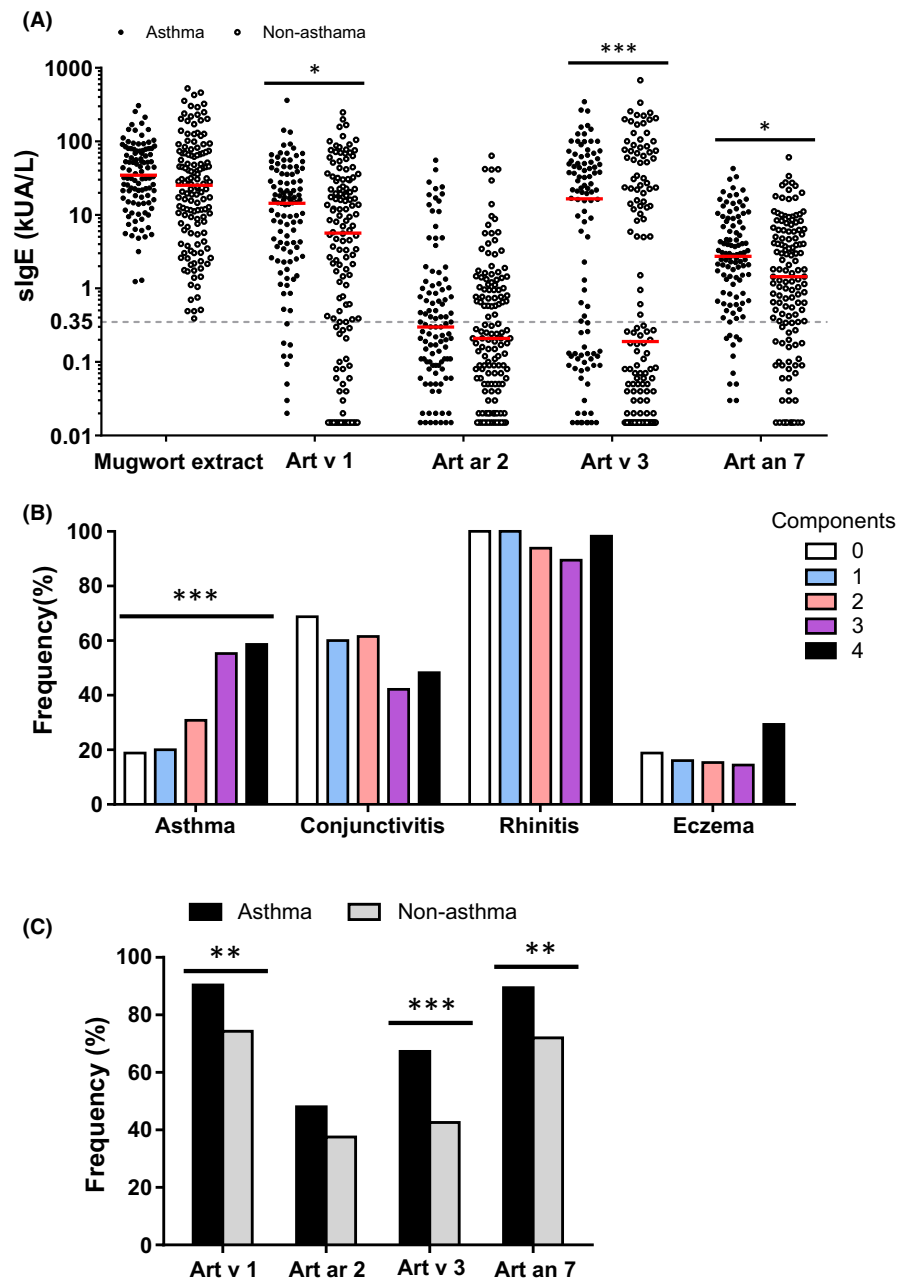
**FIGURE 2** IgE reactivity of patients from three areas. IgE levels to mugwort extract and four allergen components (A), frequency of sensitized components (B), recognized component numbers, 0 indicates none of the four tested components were positive, and 1, 2, 3, and 4 represent the number of positive individual allergens Art v 1, Art ar 2, Art v 3, and Art an 7 (C) and allergic symptoms among patients from three areas (D). SX, Shanxi province; SD, Shandong province; YN, Yunnan province. The median is shown as a red solid line, and cutoff (0.35 kUA/L) as a dashed line. Significant differences between groups are shown: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

higher pollen load in SX and possibly by different *Artemisia* species dominating in the regions.<sup>7,28</sup> Art ar 2 had the least prevalence (42%–48%) and IgE levels, being close to the 58% for Art v 2 reported in Europe.<sup>29</sup>

This is the first study to explore a possible relationship between *Artemisia* pollen molecular sensitization profiles and seasonal allergic asthma. Mugwort-allergic patients sensitized to three or four allergens had a higher risk of asthma (56.4%, OR is 2.86–3.65) than patients sensitized to two or less (31.1%). In line with this, in YN, with very low *Artemisia* pollen exposure (ratio of the total pollen count in peak month in SX, SD, and YN is 10:6:1, Figure S1) and the majority of patients being sensitized to  $\leq 2$  allergens, only one in 30 patients had allergic asthma (Figure 2D). Sensitization to a higher number of allergens is usually accompanied by a higher IgE level and potential broader cross-reactivity to allergens of pollens from not only *Artemisia*, but also from other genera in the Asteraceae family, such as *Dendranthema*, *Helianthus*, *Xanthium*, and *Ambrosia*. Art v 1 was ranked highest in inducing asthma because of its higher prevalence and OR values related to asthma. Art v 3 is the second major allergen, although its prevalence was lower than Art v 1; sensitized patients can have very high IgE levels in Northern China, and allergic reactions due to cross-reactivity to various fruits, peanut, soybean, wheat, sunflower seed have been reported in this study and previously.<sup>16,30,31</sup>

The four components used are natural proteins, so potential CCD involvement in the IgE binding cannot be excluded for those allergens known to be glycoproteins, that is, Art v 1, Art ar 2, and Art an 7. Art v 3 is a lipid transfer protein and is not glycosylated. Of the three glycoproteins, the Art ar 2 and Art an 7 carry typical CCD-like N-linked glycans,<sup>21,29</sup> whereas Art v 1 has O-linked glycans that have also been described to be recognized by IgE of some patients.<sup>32</sup> It can therefore not be excluded that both types of glycan-specific IgE antibodies play a role in recognition of the three allergens. However, we think the role of CCD-specific IgE is quite limited in Chinese patients, as in our recent paper on Art an 7,<sup>21</sup> we tested CCD in 21 patients, and found a low frequency of positivity (4/21) with low IgE levels. Moreover, our ongoing research indicates that recombinant of Art v 1 has similar IgE binding potency in mugwort-allergic patients, suggesting a minor role for IgE binding to Art v 1's O-linked glycans.

A small number of sera demonstrated significantly higher IgE binding to mugwort extract than to the sum of IgE responses against the four components tested in the present study (Figure S2). This suggests that for these patients other components are probably relevant. Similar to what was reported earlier,<sup>22</sup> Art v 5 seems to be of minor importance, and ten of these sera were negative for IgE reactivity to rArt an 5. We speculate Art v 4 and Art v 6 are most likely to be the missing components because of their broad cross-reactivity.<sup>33</sup>

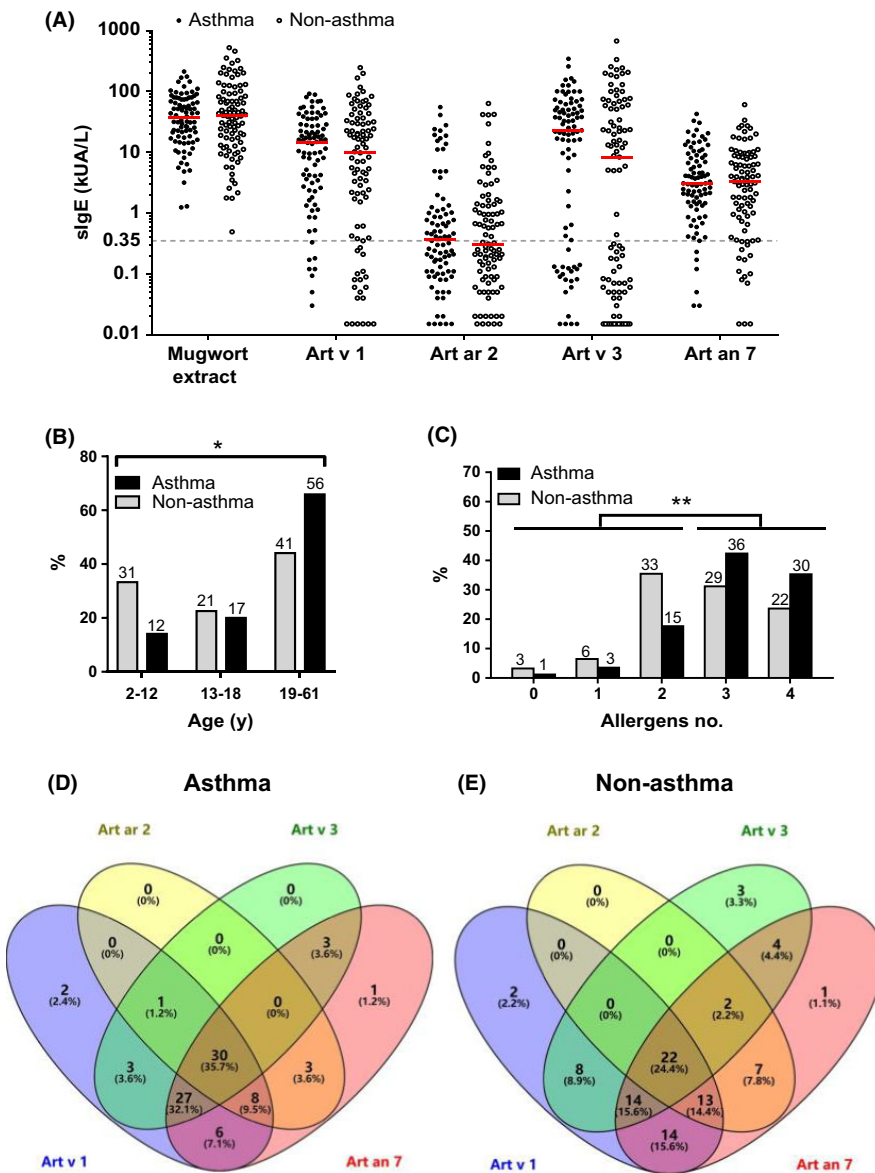


**FIGURE 3** The interrelation of asthma and non asthma symptoms with IgE levels (A), number of sensitized allergens, 0 indicates none of the four tested components were positive, and 1, 2, 3, and 4 represent the number of positive individual allergens Art v 1, Art ar 2, Art v 3, and Art an 7 (B) and component positive frequency between asthma and non asthma groups (C). Significant differences are shown \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

Because of the geographic difference associated with different *Artemisia* species distribution and pollen load in China, the pattern of *Artemisia* pollen allergen sensitization between the north and south China is dramatically different. Here, we selected two commercially available components from European (*A. vulgaris*) and two noncommercially available from Chinese mugwort (*A. argyi* and *A. annua*). This choice was made to combine convenience (commercial availability) with local relevance (dominant Chinese mugwort species). It is however unlikely that the different species display much difference in IgE binding potencies because IgE responses are expected to be highly cross-reactive between species within a genus. This assumption is based on ongoing investigations into sequence identity and variation of seven homologous allergen groups in seven *Artemisia* species. The results so far indicate that

sequence identity is very high, perhaps with the exception of group 3, that is, the LTPs that show some more heterogeneity. Therefore, it is safe to assume that the total *Artemisia* pollen load (exposure) has the largest impact on the degree of mugwort pollen allergy prevalence and its clinical phenotypes, not the possible geographic variation in mugwort species. Also a recent study provides support for this in showing that regional differences in pollen induced allergic rhinitis are associated with pollen load due to environmental and climate conditions in Inner Mongolia, China.<sup>34</sup> Inner Mongolia is north of Shanxi Province, where we enrolled 66% of patients in this research.

Most mugwort allergy patients have previously been found to first suffer mild allergic rhinitis symptoms at median age of 27.9 years, with a transition to asthma usually taking about 5 years



**FIGURE 4** IgE reactivity of asthmatic and non-asthmatic patients from Shanxi province. A, specific IgE levels to mugwort extract and four component allergens (Art v 1, Art ar 2, Art v 3 and Art an 7); B, distribution of asthmatic and non-asthmatic patients among different age groups; C, multiple allergens sensitizations in asthma and non-asthma groups, 0-none of the four tested components were positive, and 1, 2, 3, and 4 represent the number of positive individual Art v 1, Art ar 2, Art v 3, and Art an 7; and D, Venn diagrams of the interrelation of sensitized allergens in asthma and non-asthma groups; the median is shown as a red solid line, significant differences: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

**TABLE 2** Characterization of asthmatic and non-asthmatic patients from Shanxi

Patients, no. (%)	Non-asthmatic	Asthmatic	Total	P value
Patients, no. (%)	93 (52)	85 (48)	178 (100)	
Age median (range)	16 (6-59)	28 (4-61)	22 (4-61)	<0.001
Allergen positive no. (%)				
Art v 1	73 (79)	77 (91)	150 (84)	0.027
Art ar 2	44 (47)	42 (49)	86 (48)	0.779
Art v 3	53 (57)	64 (75)	117 (66)	0.01
Art an 7	77 (83)	78 (92)	155 (87)	0.075

(median age of 32.6 years),<sup>7</sup> and becoming more common after middle age. In this study, the median age was found to be 23 years for allergic rhinitis, and 28 years for allergic asthma, 5 years earlier than

previous reports. This difference in the process toward allergic asthma in teenagers may be related to higher IgE values of extract and components (Figure 1A). The main reason is perhaps the significantly higher exposure to *Artemisia* pollen due to the warmer climate (annual average temperature +1.5°C) and uncontrolled growth and spread of several dominant *Artemisia* plants in waste and marginal lands or roadsides in the suburbs and countryside in North China over the last 30 years. A comparison over 30 years in Taiyuan, Shanxi Province, showed the total *Artemisia* spp. pollen count had increased 36%, with the peak lasting 2 weeks longer.<sup>35</sup> This indicates a need to control the growth and spread of *Artemisia* plants in the wild.

In conclusion, different sensitization patterns and symptoms were observed in mugwort-allergic patients from Northern and Southern China. Sensitization to three or more mugwort allergens resulted in a higher risk of allergic asthma.



## ACKNOWLEDGMENTS

This study was supported by a grant from Hangzhou Aileji Biotech Ltd, China (2016-R1) and by the Natural Science Foundation of China (31772271). We thank Dr. Hongshan Ma for his administrative support and Ms Hong-li Chen, Jing Yang, Wen-jun Wang, Xiao-jing Du from Datong 3rd People's Hospital for their contribution in sera collection. We thank Dr. Luud Gilissen for critical comments on the draft and Dr. Shirley Burgess for correcting the English. University of Amsterdam covers the open access charge.

## CONFLICTS OF INTEREST

ZS Gao and WY Fu received a grant from Hangzhou Aileji Bio-Tech Ltd (2016-R1), China. ZS Gao received a grant from Natural Science Foundation of China (31772271). A patent to predict the potential *Artemisia* pollen allergic asthma has been applied in China (201810913872.5 pending) by ZS Gao, WY Fu, and BY Gao. The remaining authors declare that they have no relevant conflict of interests.

## AUTHOR CONTRIBUTION

ZSG, RVR, FF, GG, and HYW conceived and designed the study. WYF, JJ, LZ, SAV, and BYG purified and characterized new allergen components. YMS, MLL, FML, LYW, XFW, BHG, YF, NBT, JYZ, and HYW were involved in the clinical study of patients. WYF, SDW, YMS, XZ, YL, JJ, LZ, and BYG conducted ImmunoCAP test and data analysis. ZSG, WYF, YMS, RvR, GG, and JYZ drafted the manuscript in collaboration with all co-authors.

## ORCID

Zhongshan Gao  <http://orcid.org/0000-0001-6944-9955>

Gabriele Gadermaier  <http://orcid.org/0000-0002-4886-417X>

Fatima Ferreira  <http://orcid.org/0000-0003-0989-2335>

## REFERENCES

- Qian H, Zheng M. Prevalence of allergic disease in China. In: Gao Z, Shen H-H, Zheng M, et al., eds. *Multidisciplinary Approaches to Allergies*. Hangzhou, China: Zhejiang University Press and Springer; 2012: 3-17.
- Chen Y, Wong GWK, Li J. Environmental exposure and genetic predisposition as risk factors for asthma in China. *Allergy Asthma Immunol Res*. 2016;8:92.
- Li J, Sun B, Huang Y, et al. A multicentre study assessing the prevalence of sensitizations in patients with asthma and/or rhinitis in China. *Allergy*. 2009;64:1083-1092.
- Li J, Huang Y, Lin X, et al. China Alliance of Research on Respiratory Allergic Disease (CARRAD). Influence of degree of specific allergic sensitivity on severity of rhinitis and asthma in Chinese allergic patients. *Respir Res*. 2011;12:95.
- Wang RQ, Zhang HY. Two hundred thousands results of allergen specific IgE detection. *Chin J Allergy Clin Immunol*. 2012;6:18-23.
- Yang ZW, Gao ZS, Ma YT, Wu SD, Qiao LY. Overview of allergen sources in China. In: Gao ZS, Shen H-H, Zheng M, et al., eds. *Multidisciplinary Approaches to Allergies*. Hangzhou, China: Zhejiang University Press and Springer; 2012:59-79.
- Tang R, Sun JL, Yin J, Li Z. *Artemisia* allergy research in China. *Biomed Res Int*. 2015;2015:179426.
- Yao LN. Pollen survey by volumetric sampler in Beijing city and cross-reactivity research between ragweed and mugwort in vitro. PhD thesis, Peking Union Medical College, 2009; (in Chinese, English abstract).
- Smith M, Jäger S, Berger U, et al. Geographic and temporal variations in pollen exposure across Europe. *Allergy*. 2014;69:913-923.
- Sánchez-López J, Tordesillas L, Pascal M, et al. Role of Art v 3 in pollinosis of patients allergic to Pru p 3. *J Allergy Clin Immunol*. 2014;133:1018-1025.
- Scala E, Till SJ, Asero R, et al. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. *Allergy*. 2015;70:933-943.
- Wang HY, Gao ZS, Zhou X, et al. Evaluation of the role of IgE responses to Der p 1 and Der p 2 in Chinese house dust mite-allergic patients. *Int Arch Allergy Immunol*. 2015;167(3):203-210.
- Datema MR, Ree RV, Asero R, et al. Component-resolved diagnosis and beyond: multivariable regression models to predict severity of hazelnut allergy. *Allergy*. 2017;73:549-559.
- Hao GD, Zheng YW, Gjesing B, et al. Prevalence of sensitization to weed pollens of *Humulus scandens*, *Artemisia vulgaris*, and *Ambrosia artemisiifolia* in northern China. *J Zhejiang Univ Sci B*. 2013;14:240-246.
- Douladiris N, Savvatanos S, Roumpedaki I, Skevaki C, Mitsias D, Papadopoulos NG. A molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe: towards component-resolved management of allergic diseases. *Int Arch Allergy Immunol*. 2013;162:163-172.
- Gao ZS, Yang ZW, Wu SD, et al. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitizer. *J Allergy Clin Immunol*. 2013;131:224-226.
- Ma SK, Yin J, Jiang NN. Component-resolved diagnosis of peach allergy and its relationship with prevalent allergenic pollens in China. *J Allergy Clin Immunol*. 2013;132:764-767.
- Stringari G, Tripodi S, Caffarelli C, et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. *J Allergy Clin Immunol*. 2014;134:75-81.
- Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27(suppl 23):1-250.
- Gadermaier G, Hauser M, Ferreira F. Allergens of weed pollen: an overview on recombinant and natural molecules. *Methods*. 2014;66:55-66.
- Fu W, Gao Z, Gao L, et al. Identification of a 62 kDa major allergen from *Artemisia* pollen as a putative galactose oxidase. *Allergy*. 2018;73:1041-1052.
- Gadermaier G, Wopfner N, Wallner M, et al. Array-based profiling of ragweed and mugwort pollen allergens. *Allergy*. 2008;63:1543-1549.
- Deng S. Precision diagnosis of mugwort pollen-associated food allergy in China, PhD thesis, Peking Union Medical College, 2016.
- Movérare R, Larsson H, Carlsson R, Holmquist I. Mugwort-sensitized individuals from North Europe, South Europe and North America show different IgE reactivity patterns. *Int Arch Allergy Immunol*. 2011;154:164-172.
- Ye ST, Zhang JT, Gu RJ, et al. *A National Survey of Airborne and Allergic Pollen in China*. Beijing, China: Beijing Publisher Press; 1991.
- Gao ZS, Zhou X, Yang ZW, et al. IgE-binding potencies of three peach Pru p 1 isoforms. *Mol Nutr Food Res*. 2016;60:2457-2466.
- Pablos I, Eichhorn S, Machado Y, et al. Distinct epitope structures of defensin-like proteins linked to prolin-rich regions give rise to differences in their allergenic activity. *Allergy*. 2018;73:431-441.

28. Lombardero M, García-Sellés FJ, Polo F, et al. Prevalence of sensitization to *Artemisia* allergens Art v 1, Art v 3 and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid-transfer protein allergens. *Clin Exp Allergy*. 2004;34:1415-1421.
29. Arilla MC, Ibarrola I, Puente Y, Daza JC, Martínez A, Asturias JA. Cloning, expression and characterization of mugwort pollen allergen Art v 2, a pathogenesis-related protein from family group 1. *Mol Immunol*. 2007;44:3653-3660.
30. Ma SK, Nie L, Li H, Wang RQ, Yin J. Component-resolved diagnosis of peanut allergy and its possible origin of sensitization in China. *Int Arch Allergy Immunol*. 2016;169:241-248.
31. Wen Z, Ye S. A report of 50 patients with artemisia pollenosis and plant food allergy. *Natl Med J China*. 2002;82:626-629.
32. Leonard R, Petersen BO, Himly M, et al. Two novel types of O-glycans on the mugwort pollen allergen Art v 1 and their role in antibody binding. *J Biol Chem*. 2005;280:7932-7940.
33. Pichler U, Hauser M, Wolf M, et al. Pectate lyase pollen allergens: sensitization profiles and cross-reactivity pattern. *PLoS One*. 2015;10:e0120038.
34. Wang X, Ma T, Wang X, et al. Prevalence of pollen-induced allergic rhinitis with high pollen exposure in grasslands of northern China. *Allergy*. 2018;73:1232-1243.
35. Zhang KJ, Wang CS, Cheng NS, Wang N, Liu TQ, Wang BQ. Comparison of the result from two airborne pollen surveys conducted in Taiyuan city during the past thirty years. *Chin J Allergy Clin Immunol*. 2011;5:97-101.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Gao Z, Fu W-Y, Sun Y, et al. *Artemisia* pollen allergy in China: Component-resolved diagnosis reveals allergic asthma patients have significant multiple allergen sensitization. *Allergy*. 2018;00:1–10. <https://doi.org/10.1111/all.13597>