

Letter to the Editor

Molecular and immunological characterization of two polcalcins as novel allergens of *Artemisia sieversiana* pollen

Dear Editor,

Artemisia pollen is considered one of the leading causes of outdoor allergens in northern China.¹ Currently, *Artemisia sieversiana* pollen extracts are widely used in China for clinical skin prick tests and specific immunotherapy.² However, only five allergens have been registered, Art si 1, Art si 2, Art si 3, Art si 8, and Art si 12. Therefore, the full analysis and characterization of more potential allergens in *A. sieversiana* pollen requires further investigation.

In this study, we identified two polcalcins from *A. sieversiana* pollen designated Art si 5.0101 and Art si 5.0201; they are composed of 249 and 261 nucleotides (82 and 86 amino acids), respectively, and share 57% sequence identity (Fig. 1 A, B). Amino acid sequence comparison analysis showed that the sequence homology between Art si 5.0101/5.0201 and other polcalcin allergens was 57%–99% (Fig. 1 C). The sequence similarity between Art si 5.0101 and Art v 5 is up to 99%. Similar levels of sequence identity have been reported for other *Artemisia* species. For example, there is high amino acid sequence similarity between the same group allergens from *Artemisia annua* and *Artemisia vulgaris* pollen, particularly the 100% sequence identity in Art an 4.0101 vs. Art v 4.0101 and Art an 4.0201 vs. Art v 4.0201.³ We infer that there may be high sequence conservation among some allergen groups in *Artemisia* species. Comparatively, Art si 5.0201 shows high sequence identity with Sal k 7 (95%) and Che a 3 (90%), which belong to the Amaranthaceae family. It has long been thought that polcalcins of *Artemisia* and even *Ambrosia* should be classified together, whereas we reported a completely novel isoform of polcalcin Art si 5.0201, which is distinct from traditional polcalcins in *Artemisia*.

The Art si 5.0101 and Art si 5.0201 genes were expressed in *E. coli* BL-21 (DE3) and purified by nickel affinity and ion-exchange chromatography (see the details in Supplementary Methods). The proteins migrated as a single band with an approximate molecular weight of 10 kDa or 12 kDa (Supplementary Figs. 1A and B). Three homologous polcalcins, Art v 5, Amb a 9 and Sal k 7, were also expressed and purified (Supplementary Figs. 1C–E).

Specific IgE reactivity of the recombinant polcalcin allergens was demonstrated by ELISA using sera from 130 *Artemisia*-allergic patients (Supplementary Table 1) and 14 healthy subjects. The sensitization prevalence of Art si 5.0101 and Art si 5.0201 was

3.1% (4/130) and 3.8% (5/130), respectively (Fig. 2A). Among them, three of the patients displayed abnormally high specific IgE to the recombinant polcalcins with an OD value over 4 times the cut-off value. The reported IgE reactivity of polcalcin allergens is 5% for Bet v 4, 6% for Par j 4, 10% for Cyn d 7/Phl p 7, 11.4% for Art v 5/Amb a 9.0101 and 18% for Aln g 4.^{4–7} Moreover, the IgE reactivity of the Italian group (ranging from 21% to 28%) was much higher than that of the Austrian group (approximately 10%).⁸ Taken together, the results from similar studies suggest that the deviation of sensitization rates of polcalcins could be due to the pollen species, geographical locations, and different populations. In addition, five ELISA-positive sera to Art si 5.0101 and Art si 5.0201 were further validated by Western blot (Fig. 2 B, C), and four showed prominent bands except for the serum 31.

Pooled sera of 4 polcalcin-allergic patients were used to conduct the IgE-ELISA inhibition test between the polcalcins and the crude extract of *A. sieversiana* pollen. Approximately 60% inhibition rates were shown at the 100 µg/mL concentration of polcalcins (Supplementary Fig. 1F). The results indicated that polcalcin-specific IgE accounted for a large proportion of IgE specific to the *A. sieversiana* pollen extract in patients sensitized to polcalcins. This could be attributed to the potential properties of panallergens. For instance, both Che a 2 and Che a 3 were able to inhibit approximately 50% of the IgE binding to *Chenopodium album* pollen extract.⁹

The high degree of conservation of polcalcins implied that they may show IgE cross-reactivity. Due to the limited serum availability of four polcalcin-positive patients, we chose a large concentration of homologous polcalcins (100 µg/mL) to obtain the maximal IgE inhibition rates (Fig. 2 D). The results showed that Art si 5.0101, Art v 5, Amb a 9.0101, Art si 5.0201 and Sal k 7 could almost completely inhibit IgE binding to Art si 5.0101 (approximately 90%). Art si 5.0101 and Art v 5 showed 34% inhibition of IgE binding to Art si 5.0201, and Amb a 9.0101 showed 77% inhibition. Calcium-binding proteins are a class of cross-reactive panallergens. The reported possible IgE cross-reactive epitopes are marked with blue circles in Figure 1C (Supplementary Refs. 3–6). These cross-reactive epitopes are located in highly conserved sequences, including two calcium-binding sites and homologous exchanges (such as Glu/Asp and Ser/Thr). Accordingly, the highly conserved functional amino acid sequence ensures the cross-reactivity of polcalcins among different species.

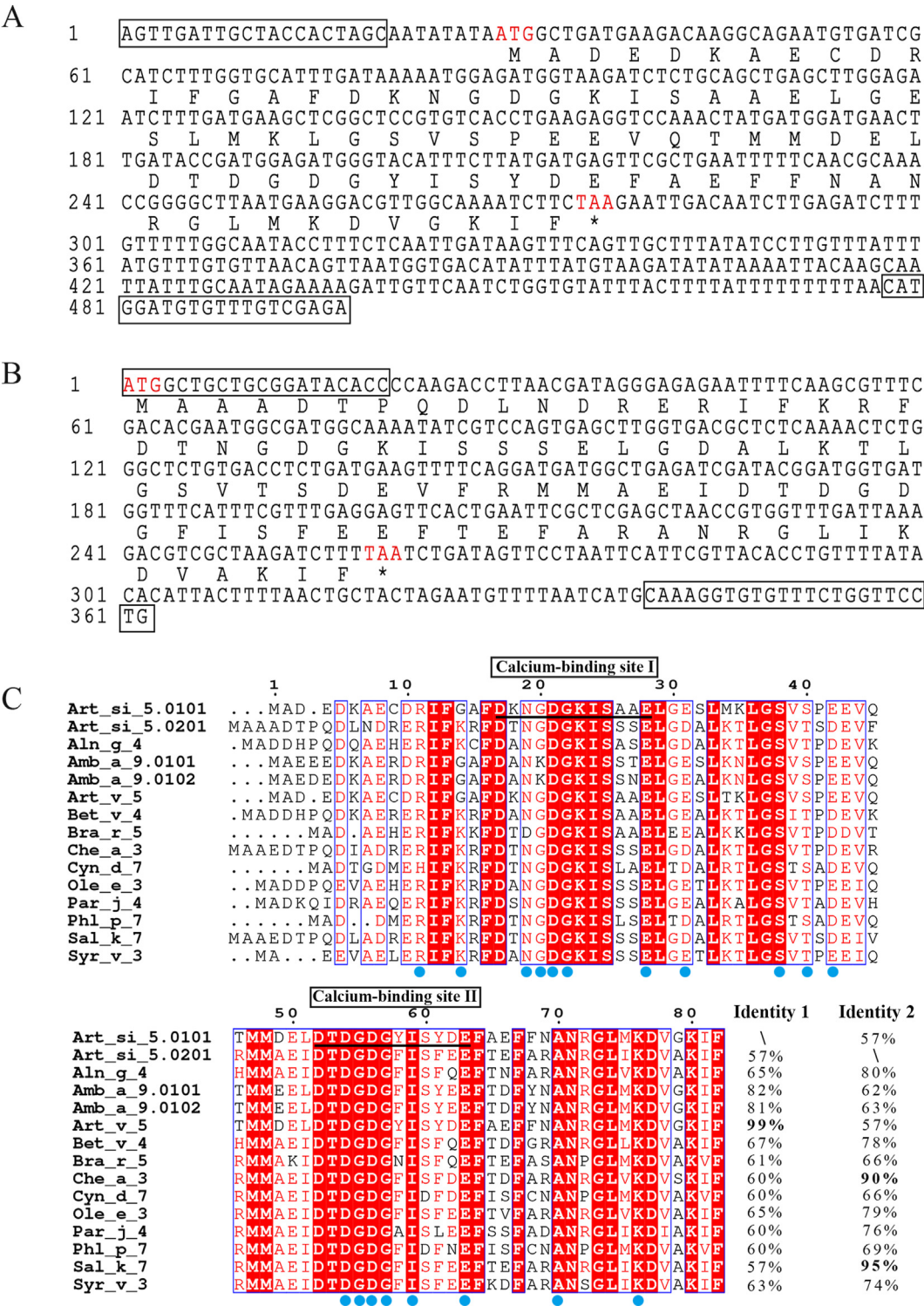


Fig. 1. Molecular characterization of Art si 5.0101 and Art si 5.0201. (A, B) Nucleic acid and amino acid sequences of Art si 5.0101 and Art si 5.0201. Initiation and termination codons are marked in red. Primers designed for PCR were framed. (C) The amino acid sequence comparison of Art si 5.0101 and Art si 5.0201 with polcalcins from different plant pollens (the sequences retrieved from WHO/IUIS Allergen Nomenclature Database: <http://www.allergen.org/>). The conserved residues are framed in blue. Identical and similar residues are indicated in white and red letters, respectively. Two calcium-binding sites are marked with black lines. The possible IgE cross-reactive epitopes of polcalcins are marked with blue circles (Supplementary Refs. 3–6). Identity 1 (Identity 2) indicates the percentage of amino acid sequence similarity between Art si 5.0101 (Art si 5.0201) and the homologous polcalcins of other species.

Further application of BAT can more accurately reflect the clinical relevance of Art si 5.0101 and Art si 5.0201. Fresh whole blood from two newly recruited *Artemisia* pollen-allergic patients (Patients A and B) was collected. Patient A showed negative

stimulation, and patient B showed obvious positive stimulation (Fig. 2 E). For patient B, when comparing the negative control and experimental groups, Art si 5.0101 and Art si 5.0201 differed significantly ($p = 0.044$ and 0.015 , respectively), with a 3.6-fold and 4.6-

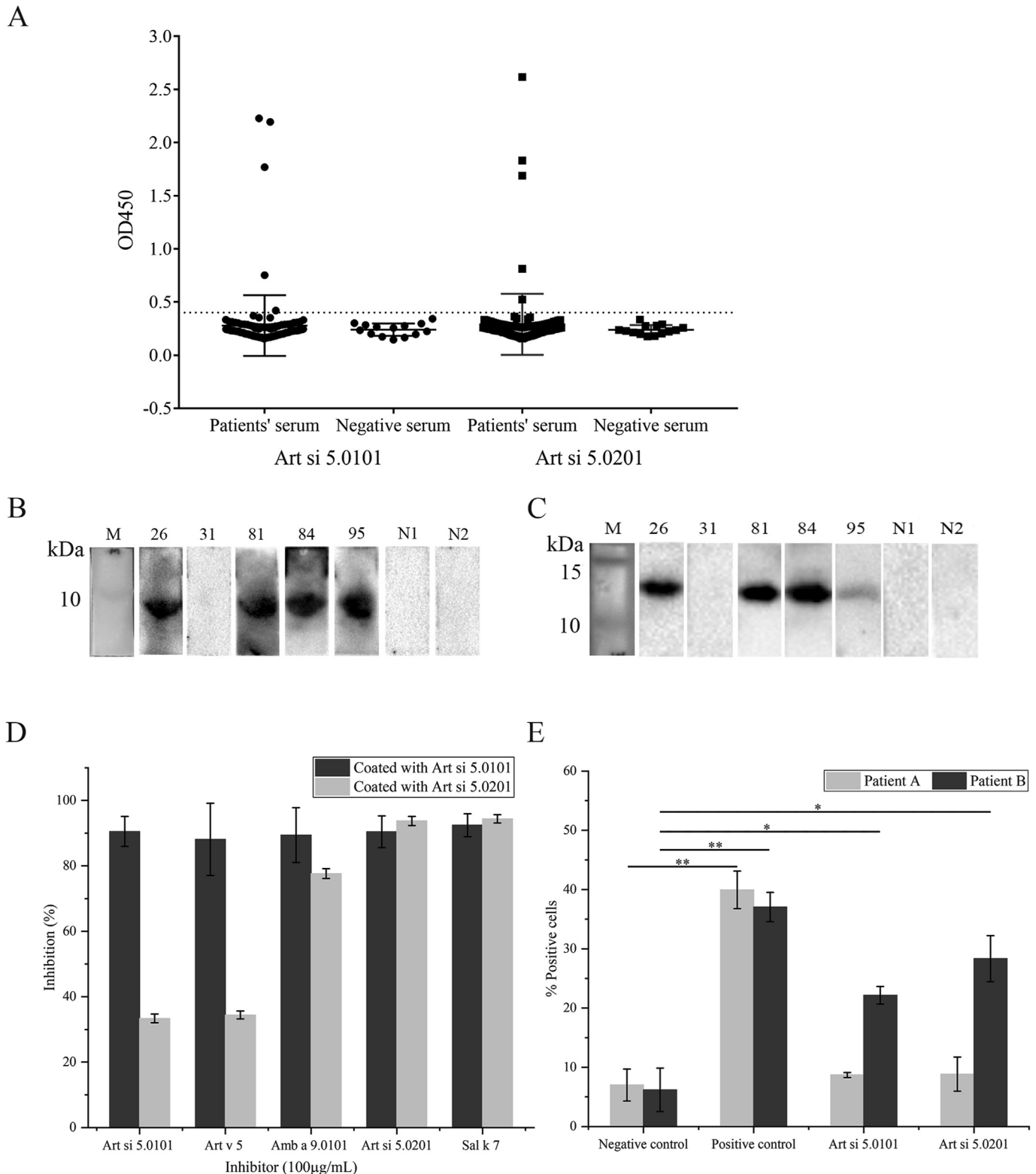


Fig. 2. IgE binding of Art si 5.0101 and Art si 5.0201. **(A)** IgE binding in ELISA. 130 *Artemisia* pollen allergic patients and 14 non-allergic individuals' sera were involved in the assay. The cutoff values were calculated as the mean absorbance value of the negative samples plus 3 standard deviations. The data are presented as mean $\pm$ SD ($n = 3$). **(B, C)** IgE binding in immunoblotting. Art si 5.0101 and Art si 5.0201 (reduced samples) were incubated with the 5 positive representative serum samples and 2 negative control serum samples, respectively. **(D)** Crossreactivity was assessed by inhibition ELISA. Inhibition assay was performed by pre-incubating pool of sera from four Art si 5.0101/5.0201 allergic patients (No. 26/81/84/95) with Art si 5.0101, Art v 5, Amb a 9.0101, Art si 5.0201 or Sal k 7 at 100 μ g/mL. The data are presented as mean $\pm$ SD ($n = 3$). **(E)** Basophil activation test in two newly recruited *Artemisia* allergy patients. The specific-IgE of *Artemisia vulgaris* in patient A was 1.68kU/L (class grade 2); the sIgE in patient B was 3.07kU/L (class grade 2). Allergen-specific basophil degranulation was analyzed by monitoring the proportion of CCR3 and CD63 positive cells. The data are presented as mean $\pm$ SD ($n = 3$). P values were determined by Dunnett's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

fold increase in the proportion of CCR3⁺CD63⁺ cells. Our present analysis illustrates that Art si 5.0101 and Art si 5.0201 are clinically relevant pollen allergens.

In summary, this study extends our understanding of the molecular and immunological properties of polcalcins in *A. sieversiana* pollen. The high degree of conservation with homologous polcalcins highlights the role of Art si 5.0101/5.0201 as potential cross-reactive markers. In addition, our results illustrate that Art si 5.0101 and Art si 5.0201 are clinically important in patients with significant IgE reactivity despite their low sensitization rates. Art si 5.0101 and Art si 5.0201 may be considered for potential desensitization vaccines appropriate for molecular-based allergen immunotherapy.

Acknowledgements

This project was sponsored by the grants from the National Natural Science Foundation of China (81871265, 81771725, U1832212); Science and Technology Research Project of the Education Department of Jiangxi Province (GJJ201532); Science and Technology Program of Health Commission of Jiangxi Province (202130624); The key research and development plan of Ganzhou Science and Technology Bureau ([2020]60–286); Ganzhou Science and Technology Bureau Guiding Science and Technology Plan Project (GZ2020ZSF002); Scientific Research Program of Health Commission of Ganzhou Municipality (2020-2-97).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2022.10.006>.

Conflict of interest

The authors have no conflict of interest to declare.

Ya-Li Cheng^{a,b,1}, Zhi-Qiang Xu^{c,e,1}, Han Wang^{e,1}, Dan-Xuan Zhu^c, Ying Zhu^{d,***}, Jin-Lyu Sun^{b,**}, Ji-Fu Wei^{a,c,*}

^a Department of Pharmacy, Jiangsu Cancer Hospital, The Affiliated Cancer Hospital of Nanjing Medical University, Jiangsu Institute of Cancer Research, Nanjing, China

^b Allergy Department, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

^c Clinical Allergy Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

^d Department of Blood Transfusion, First Affiliated Hospital of Gannan Medical University, Ganzhou, China

^e Research Division of Clinical Pharmacology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

*, **, *** Corresponding authors.

E-mail addresses: weijifu@njmu.edu.cn (J.-F. Wei), sunjinlv@pumch.cn (J.-L. Sun), pink-shadow@gmu.edu.cn (Y. Zhu).

References

1. Tang R, Sun JL, Yin J, Li Z. Artemisia allergy research in China. *Biomed Res Int* 2015;2015:179426.
2. Shi HY, Pan C, Ma TT, Chen YL, Yan WJ, Liu JG, et al. Clinical efficacy evaluation of 1-year subcutaneous immunotherapy for artemisia sieversiana pollen allergic rhinitis by serum metabolomics. *Front Pharmacol* 2020;11:305.
3. Fu W, Gao Z, Gao L, Jin J, Liu M, Sun Y, et al. Identification of a 62-kDa major allergen from Artemisia pollen as a putative galactose oxidase. *Allergy* 2018;73:1041–52.
4. Hayek B, Vangelista L, Pastore A, Sperr WR, Valent P, Vrtala S, et al. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: structural basis for calcium-modulated IgE recognition. *J Immunol* 1998;161:7031–9.
5. Wopfner N, Gruber P, Wallner M, Briza P, Ebner C, Mari A, et al. Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy* 2008;63:872–81.
6. Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *Biol Chem* 1997;272:28630–7.
7. Niederberger V, Hayek B, Vrtala S, Laffer S, Twardosz A, Vangelista L, et al. Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999;13:843–56.
8. Wopfner N, Dissertori O, Ferreira F, Lackner P. Calcium-binding proteins and their role in allergic diseases. *Immunol Allergy Clin North Am* 2007;27:29–44.
9. Barderas R, Villalba M, Pascual CY, Batanero E, Rodriguez R. Profilin (Che a 2) and polcalcin (Che a 3) are relevant allergens of Chenopodium album pollen: isolation, amino acid sequences, and immunologic properties. *J Allergy Clin Immunol* 2004;113:1192–8.

Received 18 April 2022

Received in revised form 5 October 2022

Accepted 6 October 2022

Available online 10 November 2022

¹ Ya-Li Cheng, Zhi-Qiang Xu and Han Wang contributed equally to this paper.