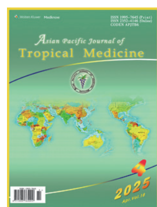

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Characterization and cross-reactivity assessment of group-29 allergens originating from *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Tyrophagus putrescentiae*

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ABSTRACT

Objective: To characterize the group-29 allergens from *Dermatophagoides* (*D.*) *pteronyssinus* and investigate their ability to cross-react with other group-29 allergens from *D. pteronyssinus* as well as those from *D. farinae* and *Tyrophagus putrescentiae*.

Methods: Der p 29, Der f 29, and Tyr p 29 cDNA sequences were amplified from total RNA isolated from *D. pteronyssinus*, *D. farinae* and *Tyrophagus putrescentiae*, respectively. Then they were cloned into the pET28a vector, expressed in Rosetta2(DE3)plysS, and purified using anion exchange chromatography. The IgE-binding rates of rDer p 29 were assessed by IgE Western blotting. The four epitopes of rDer p 29 were predicted, synthesized, and detected by IgE-ELISA. The cross-reactivity among the recombinant proteins rDer p 29, rDer f 29, and rTyr p 29 was investigated using dot blot and IgE-ELISA inhibition experiments. The allergens' physiochemical properties, amino acid sequences, and tertiary structures were also compared.

Results: Der p 29 was successfully expressed in Rosetta2(DE3)plysS as a single, 393-bp open reading frame. Western blotting showed that the purified rDer p 29 protein exhibited an high IgE-binding rate when tested on patient sera. The following four Der p 29 epitopes were predicted and synthesized: 37-45 (EP1), 57-69 (EP2), 75-80 (EP3), and 104-117 (EP4). IgE-ELISA tests on 20 *D. pteronyssinus*-positive sera yielded IgE-binding rates of 85% (rDer p 29), 80% (EP1), 55% (EP2), 40% (EP3), and 55% (EP4), respectively. The dot blot experiments further confirmed cross-reactivity among the three group-29 proteins. When used as an inhibitor, rDer p 29 demonstrated an average cross-reactive inhibition rate of 49.7% against rDer f 29 and 54.4% against rTyr p 29. When rTyr p 29 was used as an inhibitor, it showed an average cross-reactive inhibition rate of 56.3% against rDer f 29.

Conclusions: A recombinant protein, rDer p 29 with strong allergenicity was produced. Moreover, it was found that rDer p 29 cross-reacted with rDer f 29 and rTyr p 29, due to their highly homologous sequences and structures. These findings highlight the importance of considering cross-reactivity when diagnosing and treating allergic diseases.

Summary

Question: Why does cross-reactivity occur among different mites within the same allergen group?

Findings: Due to their highly homologous sequences and structures, the recombinant allergens rDer p 29 cross-reacted with rDer f 29 and rTyr p 29.

Meaning: Identifying cross-reactivity among homologous allergens and those linked to other allergic diseases is key to enhancing diagnostics and treatment.

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1. Introduction

The incidence of allergic disorders, including atopic dermatitis, asthma, allergic rhinitis *etc*., has increased over the past 50 years. The global prevalence of allergic disorders is predicted to reach 4 billion cases by 2050[1]. It is well established that exposure to aeroallergens, such as house dust mites (HDMs), pollen, mold, and animal dander, carries a strong risk of sensitization and the development or exacerbation of atopic diseases. The HDMs are one of the most common allergen sources in the world; they are the causal agent in >90% of the cases of atopic dermatitis in the Asian population[2]. A large multicenter study on the prevalence patterns of serum-allergen-specific IgE sensitization among 44 156 patients (from 52 cities and 26 provinces across China) with allergic symptoms, found that HDMs were the allergens associated with the highest prevalence of sensitization in mainland China[3]. According to the study, Southern China had the highest (40.79%) prevalence of HDM sensitization, and Northeast China had the lowest (11.21%) in mainland China. *Dermatophagoides* (*D.*) *pteronyssinus* and *D. farinae* are the two most common pyroglyphid species associated with allergic diseases. *Blomia tropicalis*, a storage mite, is also found in high numbers in Asian homes and is also regarded as an important allergy-inducing mite. *Tyrophagus* (*T.*) *putrescentiae* (family Acaridae), a ubiquitous storage mite species, is considered the most clinically relevant allergy-causing mite species worldwide[4–6].

Traditionally, HDM extracts have been used to guide allergy diagnosis and treatment. However, because the extract contains a mixture of allergens, as well as non-allergy-inducing and non-toxic materials, it is difficult to define the absolute potency of each allergen. Moreover, using the extract during immunotherapy can elicit adverse reactions, which can be life-threatening. To date, 40 groups of HDM allergens have been identified and registered by the World Health Organization and International Union of Immunological Societies Allergen Nomenclature Sub-Committee. However, the HDM extract has only been standardized for the Der p 1 and Der p 2 allergens of *D. pteronyssinus*. The absence of some important components of the HDM from these standardized extract preparations may contribute to missed or inaccurate diagnoses[7]. Six HDM allergens, comprising the major allergens Der p 1, Der p 2, and Der p 23, and the mid-tier allergens Der p 5, Der p 7, and Der p 21, represent the most clinically relevant HDM allergens. These allergens can diagnose 98%-99% of HDM-allergic patients and hold promise for application in allergen-specific immunotherapy[8].

Despite recent advances in allergen selection, current diagnostic methods that rely on crude HDM extracts are suboptimal, necessitating the development of more effective diagnostic and treatment methods. Component-resolved diagnostics (CRD) is a new tool that aims to detect IgE-mediated sensitization against individual and relevant allergens. CRD uses single molecules or panels of allergens to discriminate between primary sensitization and cross-reactivity in patients with double or multiple positivity in diagnostic tests that use whole extracts. This strategy enables the selection of the most suitable agents for specific immunotherapy (SIT), which avoids the harmful side effects associated with unnecessary SIT. The identification of cross-reactive, recombinant pairs of allergens derived from different species may further enhance the diagnostic performance of CRD. CRD may be particularly useful for patients with negative allergy tests and a proven history of a previous systemic reaction, including those with mast cell disorders, who could benefit from SIT. The different sensitization profiles identified in HDM-allergic patients could be associated with a greater risk of SIT failure or treatment side effects. With the advent of recombinant DNA technology, recombinant allergens and their synthetic peptides may offer a potentially safer and more effective approach to allergy diagnosis and treatment[9]. The use of purified and/or recombinant allergens increases the accuracy of individual sensitization profiles in allergic patients, resulting in a more individualized, "tailor-made" form of immunotherapy. Recently, cyclophilins were demonstrated as a pollen panallergens inducing IgE-mediated cross-reactivity between homologous allergens from phylogenetically distant species[10]. The group-29 allergens in mites were identified as cyclophilins[11]. Our primary aim was to clone and express copy (c)DNA sequences encoding the group-29 allergens from *D. pteronyssinus*, *D. farinae* and *T. putrescentiae* to evaluate their allergenicity. We used an IgE enzyme-linked immunosorbent assay (ELISA) inhibition assay to show that the sequence similarities among Der p 29, Der f 29, and Tyr p 29 caused their cross-reactivity. Thus, our findings suggest that if a person were allergic to rDer p 29, they would also have an IgE reaction to rDer f 29 or rTyr p 29. This research highlights the clinical importance of identifying cross-reactivity among homologous HDM allergens and those responsible for other allergic diseases.

2. Methods

2.1. Cloning and sequencing of Der p 29

Total RNA was extracted from *D. pteronyssinus* using the RNAiso Plus Kit (TaKaRa Biotech Co. Ltd, Dalian, China; Catalogue No. 9108), digested with RNase-free recombinant DNase I (TaKaRa, Code No. 2270A), and was used as a template for

synthesizing cDNA using the TaKaRa PrimeScript™ RT-PCR Kit (TaKaRa, Code No. RR014A). The cDNA encoding Der p 29 was amplified by PCR using the TaKaRa Tks Gflex DNA Polymerase (TaKaRa, Code No. R060A), the forward primer 5'-AATATCTACGCGGTTTGTGTTTGA-3' and the reverse primer 3'-TTTTTCGGAAGGATATATTGAGTT-5'. This PCR product was used as a template for a second PCR amplification, using forward primer 5'-TAATCATCATCATTCTTATCATCG-3' and the reverse primer 5'-TTTTTCGGAAGGATATATTGAGTT-3'. After purification and sequencing, the second PCR product was used as template for amplification, using the forward primer 5'-AATGGGTCGCGGATCCATGTCTTGGCAATCATATGTC-3' and the reverse primer 5'-TGCTCGAGTGCGGCCGCTCAATAATTATTGGATCTCAA-3'. After being purified with the MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (TaKaRa, Code No. 9762), the product was inserted into the pET28a(+) vector, which was digested with *Bam*H I/*Not* I and thermally transfected into the JM109 *Escherichia coli* competent cells (TaKaRa, Code No. 9052). The coated plate was cultured overnight at 37 ℃. Plasmids were extracted from positive bacterial colonies and were sequenced using the forward primer 5'-AATGGGTCGCGGATCCATGTCTTGGCAATCATATGTC-3' and the reverse primer 5'-TGCTCGAGTGCGGCCGCTCAATAATTATTGGATCTCAA-3'. Once the sequencing results confirmed that the clones were identical to their corresponding PCR products, the plasmids were selected for protein expression.

2.2. Expression and purification of the recombinant rDer p 29, rDer f 29, and rTyr p 29 proteins

The constructed prokaryotic expression plasmid pET28(+)-Der p 29 was transformed into Rosetta2(DE3)plysS competent cells, which were plated out and cultured overnight at 37 ℃. A single colony was used to inoculate an LB flask and cultured for 16 hours. Next, 10 mL of the culture solution was transferred to a 2 L flask containing 500 mL LB and cultured at 37 ℃ at 200 rpm until an OD_{600} value of ~0.6 was reached. Cells were induced with 1 mM IPTG and cultured for 4 hours. The bacteria (OD_{600}=2.0) were collected by centrifugation, washed with PBS, resuspended in 320 μL of PBS, and fragmented using ultrasound. The thallus fragmentation solution was separated by centrifugation at 12 000 rpm for 5 minutes. Each 8 μL sample of total protein, supernatant, and precipitate (OD_{600}=0.05) was heated at 95 ℃ for 10 minutes with 2 μL 5× SDS loading buffer. SDS-PAGE was performed to evaluate the expression of the target protein in each fraction.

Recombinant proteins were collected by ultrasonic fragmentation and bacterial centrifugation. Briefly, 2.2 g of bacteria were resuspended in 25 mL of sonication buffer and sonicated at 180 W, 4 ℃ for 10 minutes. And 27 mL of the sonicated suspension was centrifuged at 12 000 rpm, 4 ℃ for 30 minutes. The supernatant was collected and filtered using a 0.45 μm 1 000 mL vacuum filter/storage bottle system. The recombinant proteins in the filtrate were purified using the GE HiTrap TALON crude 5 mL and TALON Superflow (Code No. 28-9537-66) columns. A total of 50 mL of sterilized MilliQ H_2O and 50 mL buffer A were used (both at 10× the column volume) to balance the resin at a flow rate of 1 mL/minute. The sample was then loaded at a flow rate of 0.5 mL/min. After protein adsorption, 50 mL of buffer A (at 10× the column volume) was used to wash the resin at a flow rate of 1 mL/min. A total of 50 mL buffer A and 50 mL buffer B were used to elute the protein at a flow rate of 1 mL/minute. The purified target proteins were collected and dialyzed in PBS three times. After dialysis, 0.5 μL and 1 μL protein samples were loaded into a polyacrylamide gel for SDS-PAGE; BSA was used as a positive control. Quantitative analysis of the concentrated samples was performed using ImageMaster 1D (version 3.0) software.

The recombinant proteins rDer f 29 and rTyr p 29 were prepared in the same way as rDer p 29, after their genes were amplified from the total RNA of *D. farinae* or *T. putrescentiae* based on GenBank nos. KM010010.1 and KX060615.1, respectively.

2.3. Western blotting detection of IgE binding to recombinant proteins

Sera (Group 1) were collected from 20 children (8 female, 12 male) aged 6 months to 10 years. 16 of the children were allergic to the *D. pteronyssinus* extract; among them, 12 had bronchial asthma, three had a chronic cough with an unknown cause, and one had cough-variant asthma. The remaining four children were healthy.

Specific IgE were detected by ImmunoCAP technology (UniCAP 1000, Pharmacia Diagnostics, Uppsala, Sweden). About 1 μg of recombinant protein was diluted in PBS to a total volume of 16 mL. SDS-PAGE was performed at 95 ℃ for 10 minutes. The serum was diluted 1:10 and used as primary antibody, while 1: 4 000 dilution of mAb to Human IgE (HRP) was used as the secondary antibody to detect serum-specific IgE binding rates.

2.4. Epitope prediction of rDer p 29 amino acid sequences

2.4.1. Epitope prediction and artificial synthesis

The amino acid sequences of potential B cell epitopes of Der p 29 were analyzed using the online software BcPred, ABCpred, AAP, FBCPred. Four peptide segments were selected for artificial synthesis.

2.4.2. IgE-ELISA binding test for artificially synthesized epitope peptides

The recombinant allergen and four synthetic peptides were incubated in carbonate buffer (pH 9.0) at 4 ℃ overnight and then treated with PBS containing 5% BSA at 37 ℃ for 2 hours; the sera from the patients allergic to HDMs and the healthy subjects (negative controls) were diluted at a ratio of 1:10 and incubated at 37 ℃ for 1 hour. The samples were then incubated with biotin-conjugated mouse anti-human IgE and streptavidin-conjugated horseradish peroxidase preparations at 37 ℃. TMB was then used for color development. After 10 minutes, 2 M sulfuric acid was added to terminate the reaction. Absorbance was measured at a wavelength of 450 nm.

Twenty serum samples (Group 2) taken from patients allergic to HDMs and six serum samples taken from healthy subjects were used in the epitope peptide IgE-ELISA assay. The mean IgE value obtained from a negative control plus three standard deviations (SDs) was used as a cut-off value for a positive IgE signal (Supplementary Table 1).

2.5. Evaluating cross-reactivity among rDer p 29, rDer f 29, and rTyr p 29

2.5.1. Dot blot

The dot blot method was used to verify cross-reactivity among the three recombinant proteins: rDer p 29, rDer f 29, and rTyr p 29. rDer f 29 (5 μg) was applied onto a nitrocellulose membrane, which was then dried for 30 minutes and blocked with milk for 2 hours. Next, 5 μg of rDer p 29 or rTyr p 29, acting as an inhibitor, was added to 100 μL of HDM-allergic serum diluted at a ratio of 1:5. After the suppressed serum was incubated at 37 ℃ for 2 hours, it was applied onto the pre-prepared NC membrane. After an overnight incubation, the NC membrane was washed three times with TBST and incubated at 37 ℃ for 2 hours with HRP-conjugated IgE antibodies. After another washing step, the membrane was treated with ECL luminescent liquid A and liquid B (combined at a ratio of 1:1). Finally, the NC membrane was analyzed on the imager.

In the test, positive serum samples were obtained from eight patients (three male, five female) with HDM-induced allergic rhinitis, with an average age of (34.4±6.2) years. Negative serum samples from two patients (one male, one female) with an average age of (8.5±0.7) years were used as negative controls. Allergen-specific IgE was detected using AlleisaScreen/AllergyScreen Test Kit (Mediwiss Analytic, Moers).

2.5.2. IgE-ELISA inhibition experiment t

96 well plates were coated with 100 μL/well of 5 mg/mL rDer f 29 and incubated overnight at 4 ℃. The plates were blocked with milk for 2 h. Next, 100 μL of 5 mg/mL of rDer p 29 or rTyr p 29 (used as inhibitors) was combined with 100 μL of HDM-positive serum (diluted 1:5), and 200 μL of the mixture was added to each well of the plate. After an overnight incubation, the plates were washed three times with TBST, and incubated with 100 μL/well of HRP-conjugated IgE at 37 ℃ for 2 hours. Next, 100 μL/well of TMB was added, and the color was allowed to develop for 30 minutes. The OD values were measured at 450 nm after the addition of the stop solution. The inhibition rate was calculated using the following formula: (positive serum OD value before inhibition-corresponding positive serum OD value after inhibition) / (positive serum OD value before inhibition-average negative serum OD value).

Positive sera were obtained from 10 patients with allergic rhinitis (four male, six female), with an average age of (32.7±6.5) years. Negative sera were obtained from three healthy subjects (two male, one female) with an average age of (7.7±1.5) years. Specific IgE was detected using the AlleisaScreen/AllergyScreen Test Kit (Mediwiss Analytic, Moers). The negative sera were selected from individuals without a history of atopy and with grade 0 allergen-specific IgE levels.

2.6. Comparison of Der p 29, Der f 29, Tyr p 29 protein sequences and physicochemical properties

The amino acid sequences of Der p 29, Der f 29, and Tyr p 29 were compared using the ClusterW multiple sequence alignment method and ESPript 3.0. The physicochemical properties of the proteins were analyzed using the ProtParam tool.

2.7. Ethical approval

This study was approved by the Human Ethics Committee of the Affiliated Children's Hospital of Jiangnan University (Wuxi, China; approval number: WXCH2024-06-094, approval date: June 5, 2024) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before enrollment.

3. Results

3.1. Generation of rDer p 29 and its binding to IgE

The prokaryotic expression plasmid pET28a(+)-Der p 29 was constructed by cloning in cDNA encoding Der p 29, which was amplified from total RNA isolated from *D. pteronyssinus* into pET-28a. After sequencing, a single 393-bp open reading frame was identified (Figure 1A), which was given GenBank Accession No. KY659243.1. The open reading frame encoded a 14.305-kDa protein composed of

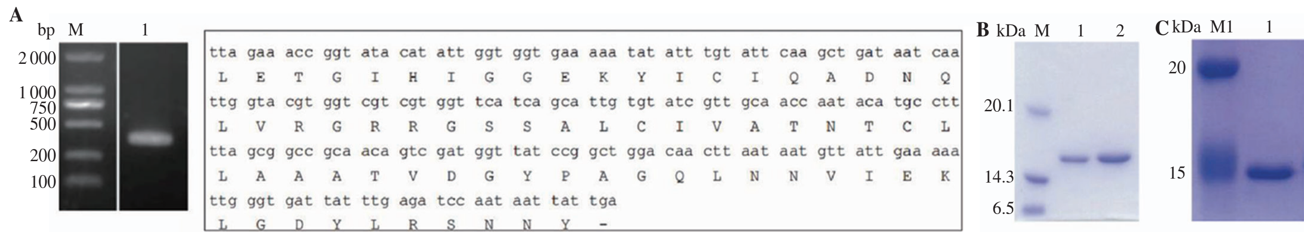

Figure 1. Production of recombinant allergen rDer p 29. (A) Cloning and sequencing of Der p 29. 1% agarose gel electrophoresis analysis of 5 µL of PCR product: lane M, DL2 000 DNA marker; lane 2, 5 µL PCR product. Analysis of the recombinant protein rDer p 29 by SDS-PAGE and Western blotting after purification. The sequence of the protein Der p 29 is shown on the right. (B) SDS-PAGE analysis of rDer p 29: lane M, protein marker (broad); lane 1, 0.5 µL purified rDer p 29; lane 2, 1.0 µL purified rDer p 29. (C) Western blotting analysis of rDer p 29: lane M1, precision plus protein standards; lane 1, 1 µL purified protein.

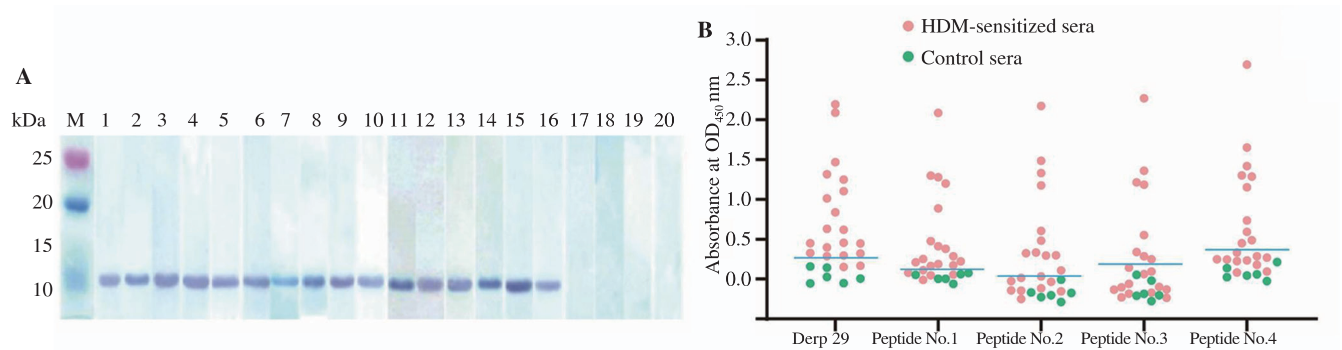

Figure 2. IgE-binding of rDer p 29 and its derived four epitope peptides. (A).Western blotting analysis of IgE binding to rDer p 29. Lane M: molecular weight standards; lanes 1-16, positive sera (against *D. pteronyssinus* extract); lanes 17-20, negative sera (against *D. pteronyssinus* extract). (B). IgE-ELISA results for Der p 29 and its four epitope peptides.

Table 1. Amino acid sequences of the Der p29 epitopes predicted by BcPred, ABCpred, AAP, and FBCPred software.

Prediction servers	Epitope prediction (Peptide, aa)	Confirmed epitopes (Peptide, aa)
BcPred	29-48,50-69,104-123	37-45DDKKISPKE
ABCpred	12-27,27-42,33-48,42-57,72-87,84-99,103-118	57-69PNGFLETGIHIGG
AAP	37-56,104-123	75-80IQADNQ
FBCPred	2-15,32-45,57-70,104-117	104-117ATVDGYPAGQLNNV

130 amino acids; the theoretical isoelectric point (pI) of the protein was 6.06. The purified recombinant protein (rDer p 29) produced a band at ~15 kDa on the SDS-PAGE gel (Figure 1B) and Western blotting (Figure 1C). IgE Western blotting showed that rDer p 29 interacted with the *D. pteronyssinus*-positive sera of all the 16 HDM-allergic pediatric patients (Figure 2A).

3.2. Epitope prediction and specific IgE detection

Four types of epitope prediction software were used to predict potential Der p 29 epitopes. BcPred predicted epitopes at residues 29-48, 50-69, 104-123; ABCpred predicted epitopes at residues 12-27,27-42,33-48,42-57,72-87,84-99,103-118; AAP predicted epitopes at residues 37-56,104-123; and FBCPred predicted epitopes at residues 2-15,32-45,57-70,104-117. From these results, four epitopes, located at residues 37-45, 57-69, 75-80, and 104-117, were selected and synthesized: DDKKISPKE (EP1), PNGFLETGIHIGG (EP2), IQADNQ (EP3), and ATVDGYPAGQLNNV (EP4) (Table 1).

Using rDer p 29, EP1, EP2, EP3, and EP4 in the IgE-ELISA experiments produced cutoff absorbance (at 450 nm) values of 0.792, 0.663, 0.579, 0.708, and 0.810, respectively, which corresponded to positive rates of 85%, 80%, 55%, 40%, and 55%, respectively. The absorbance values of most of the experimental groups were above the cutoff value (Figure 2B).

3.3. Cross–reactivity among rDer p 29, rDer f 29, and rTyr p 29

3.3.1. Dot blot

Next, we evaluated the extent of cross-reactivity among rDer p 29, rDer f 29, and rTyr p 29 using dot blotting. In these experiments,

Figure 3. Assessment of cross-reactivity among rDer p 29, rDer f 29, and rTyr p 29. (A) Dot blot assessment of cross-reactivity among rDer p 29, rDer f 29, and rTyr p 29. The dots of lanes 1-8 were coated with rDer f 29, while rDer p 29 and rTyr p 29 were used as inhibitors. (B) IgE-ELISA assessment of cross-reactivity inhibition among rDer p 29, rDer f 29, and rTry p 29 when rDer p 29 was used as an inhibitor. (C) Amino acid sequence alignment of Der p 29, Der f 29, and Tyr p 29.

rDer f 29 was used as the bait rDer p 29 and rTyr p 29 were used as the inhibitors. The addition of rDer p 29 or rTyr p 29 to rDer f 29 lightened the color of the dot, indicating that the inhibitor bound to the IgE-positive serum; thus, there was cross-reactivity between rDer f 29 and rDer p 29 or rTyr p 29 (Figure 3A).

3.3.2. IgE-ELISA inhibition experiment

When rDer p 29 was used as an inhibitor, the proportion of rDer f 29 inhibited by >50% was 50% (5/10), with an average inhibition rate of 49.7%. Meanwhile, the proportion of rTyr p 29 inhibited >50% was 70% (7/10), with an average inhibition rate of 54.4%. When rTyr p 29 was used as an inhibitor, the proportion of rDer f 29 inhibited by >50% was 70% (7/10), with an average inhibition rate of 56.3%. These two tests revealed that there was strong cross-reactivity among the three recombinant proteins (Figure 3B).

3.4. Comparison of Der p 29, Der f 29, and Tyr p 29 amino acid sequences

According to the bioinformatics analysis results, the sequence identity of the three proteins reached 92.62%, with only a few amino acid differences among them (Figure 3C).

4. Discussion

The first cDNA clone for Der p 1 was sequenced and identified in 1988[12], while the most recent group-41 allergen, Blot 41 was created in 2023 by the World Health Organization and International Union of Immunological Societies Allergen Nomenclature Sub-Committee. The molecular and immunological characterization of allergens has led to the development of recombinant allergen- and peptide-based approaches, which will help advance the prevention, diagnosis, and treatment of IgE-associated allergies[13,14].

In 2013, a proteomic approach was used alongside the conventional pure antigen discovery method to identify Der f 29, a cyclophilin, in the sera of patients allergic to HDMs[15]. In previous transcriptomics analyses, we obtained mRNA for a protein similar to Der f 29[16]. In the present study, we generated the recombinant version of Der p 29 by amplifying cDNA encoding Der p 29 and expressing in Rosetta2(DE3) plysS. After purifying rDer p 29 on a GE HiTrap TALON crude column, we investigated its IgE-binding rates by IgE-ELISA (85%; 17/20) and IgE western blotting (100%, 16/16) using *D. pteronyssinus*-positive and -negative human sera. Due to limited sample availability, the rates obtained are not representative of the true positive rates for this allergen in the whole population; however, both IgE-ELISA and IgE western blotting demonstrated good immunoreactivity and specificity for our rDer p 29.

The allergen-specific IgE specifically targets and binds to epitopes, which triggers the activation of immune cells, the release of inflammatory mediators, and the onset of clinical symptoms. Identifying epitopes is essential for the accurate monitoring of allergic responses and the development of appropriate diagnostic tools. Antigenic epitopes can be mapped using biochemical experiments and/or computational prediction methods; however, the former is often costly, time-consuming, and requires specialized laboratory equipment. Thus, *in silico* epitope discovery approaches are becoming increasingly popular. Using a variety of publicly available prediction tools, we benchmarked the allergenic IgE and T-cell epitopes of Der p 29. BcPred, ABCpred, AAP, and FBCPred tools were collectively used to design four Der p 29 epitopes, which were subsequently synthesized artificially and tested using *D. pteronyssinus*-positive sera. The IgE-ELISA results revealed that the binding rates for the four peptides ranged from 40% to 80%, validating the reliability of our bioinformatics approach. EP1 (DDKKISPKE) had the highest binding rate (80%). Future studies with a larger sample size are needed to further validate the diagnostic value of EP1.

IgE cross-reactivity is often recognized when allergic symptoms arise following exposure to a previously unencountered allergen source. However, IgE cross-reactivity may be clinically manifesting or irrelevant. IgE binding to cross-reactive, clinically irrelevant allergens results in false-positive results on *in vitro* diagnostic tests. However, unidentified sources of cross-reactive allergens of clinical relevance may lead to unintentional exposure and allergic reactions, posing a health risk to the affected individuals. The cross-reactivity between mite allergens and other allergens is due to the presence of common proteins such as tropomyosin, glutathione *S*-transferase, arginine kinase, and hemocyanin[17,18]. Cross-reactivity among proteins derived from the same or different mite species is an even more common phenomenon. Liu *et al*. showed that 70.14% of samples from 1 497 allergenic patients were co-sensitized to Blot, Der p, and Der f, with cross-reactivity detected between *Blomia tropicalis* and two *Dermatophagoides* species[19]. In the present study, a combination of IgE dot blot and IgE-ELISA inhibition experiments was used to show cross-reactivity among the family 29 allergens rDer f 29, rDer p 29, and rTyr p 29. The study of allergen cross-reactivity is key to understanding the allergic response of patients so that they can receive the most suitable treatment options. The *in vitro* diagnosis and management of allergies is often hampered by the presence of cross-reactive allergens.

It was suggested that a sequence identity >70% between two homologous allergens, such as that between Der p 1 and Der f 1, and that between Der p 2 and Der f 2, may contribute to allergen cross-reactivity[20]. It should be noted that although both the Blot 1 and Der p 1 allergens belong to group 1, their cross-reactivity is limited due to their relatively low sequence homology (53% nucleotide and 34% amino acid similarity)[21]. Our study highlighted the cross-reactivity between Der p 29 and its homologous allergens. The sequence consistency of the rDer p 29, rDer f 29, and rTyr p 29 proteins obtained by the Clustalz W multiple sequence alignment method reached 92.62%, greatly exceeding the 70% threshold. Moreover, these proteins had similar physicochemical properties and spatial structures.

In summary, we obtained the recombinant allergens rDer f 29, rDer p 29, and rTyr p 29, demonstrated their immunologic cross-reactivity due to highly homologous sequences, physicochemical properties and spatial structures, and concluded that the group-29 allergen as mite panallergen. Therefore, the component-resolved diagnosis should include this group of allergens. A limitation of this study is that the diagnostic value of the identified epitope needs to be further investigated in a larger population.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

JNC and YZ contributed to writing the original draft, data curation, data analysis and investigation. YNR, KDW, LXZ, DMZ, LYC, QC, YFL contributed to data analysis and methodology. YBC and CLH contributed to conceptualization, reviewing, and editing the manuscript.

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