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ORIGINAL ARTICLE

IgE-sensitization and cross-reactivity of Der f 23 and Der p 23 in Korean patients with allergy

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Abstract

Background: House dust mites (HDM) are a major source of allergens, and more than 30 HDM allergens have been identified to date. Recently, Der p 23 was reported to be a major allergen that is related to the severity of allergic responses.

Objective: This study aimed to characterize IgE-sensitization and cross-reactivity between Der f 23 and Der p 23 in Korean patients with allergy.

Methods: We produced recombinant Der f 23 and Der p 23 using a yeast *Pichia* expression system. The IgE binding activity of Der f 23 and Der p 23 was evaluated by enzyme-linked immunosorbent assay (ELISA) using sera from 194 Korean HDM-sensitized patients. The cross-reactivity between Der f 23 and Der p 23 was then assayed using competitive ELISA.

Results: Among the 194 HDM-allergic patients, IgE reactivity to rDer f 23 and rDer p 23 was observed in 45.36% (88/194) and 43.81% (85/194) of the samples, respectively. Competitive ELISA with pooled serum from 10 patients revealed that rDer p 23 inhibited 86.1% of the rDer f 23 IgE reactivity and rDer f 23 inhibited 61.1% of the rDer p 23 IgE reactivity.

Conclusion: Group 23 HDM allergens, Der f 23 and Der p 23, show moderate sensitization, with 45.36% and 43.81% of Korean patients with allergy reacting to them, respectively. Significant IgE cross-reactivity was observed between the two allergens. These findings can facilitate the development of component-resolved diagnosis and allergen-specific immunotherapy in the future.

Key words: house dust mite, Der f 23, Der p 23, cross-reactivity, recombinant protein

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Introduction

Allergic asthma, allergic rhinitis, and atopic dermatitis are the most common allergic diseases. They are considered major health problems worldwide and have a substantial impact on the social lives of patients.[1] House dust mites (HDM) are an important source of allergens.[2] The two predominant species, *Dermatophagoides farinae* and *D. pteronyssinus*, produce over 40 allergens with various biochemical functions, including the most well-known allergens, group 1 (Der p 1 and Der f 1) and group 2 (Der p 2 and Der f 2).[3] Der p 23, an intestinal-derived peritrophin,

which is present in the outer membrane of the mite feces, has been identified as a major allergen.[4,5] Sensitization to Der p 23 is associated with a high risk of atopic dermatitis[6] and increased prevalence of allergic rhinitis symptoms.[7] The currently identified HDM allergens are grouped by molecular profile and predicted activity.[3] For example, group 1 allergens (Der p 1 and Der f 1) are cysteine proteases that share approximately 81% sequence identity and exhibit antigenic cross-reactivity.[8] Der p 23 is a small globular protein with a carbohydrate-binding domain and is stabilized by two disulfide bonds, which are structurally similar to those in other allergens such as Blo t 12.[9]

Although it has been demonstrated that Der p 23 is an important trigger for HDM allergy, IgE reactivity to Der p 23 and Der f 23 has not yet been investigated in Korean patients with allergy. Therefore, in this study, we aimed to characterize the serum IgE binding activity of recombinant Der f 23 And Der p 23 using sera from 194 Korean HDM-sensitized patients. Additionally, we examined the cross-reactivity between these two recombinant allergens using competitive enzyme-linked immunosorbent assay (ELISA) to identify the similarity between them. Thus, the overall aim of the study was to investigate the potential usefulness of these two recombinant allergens for mite component-resolved diagnosis and immunotherapy.

Materials and Methods

D. farinae-sensitized patients

Overall, 194 serum samples were obtained from patients attending the Allergy-Asthma Clinic at Severance Hospital, Yonsei University College of Medicine in Seoul, Korea. The diagnosis of allergy was based on any history of allergic reactions and skin prick testing. Sera from the patients were tested for IgE antibodies specific for HDM (*D. farinae*) allergens using the ImmunoCAP system (Phadia, Uppsala, Sweden) and ELISA. Sera from the 194 HDM-sensitized participants (males/females, 103:91; average age, 25.4 years; age range, 4–67 years) and 20 healthy controls were used to assess the IgE reactivity of the recombinant proteins. Serum samples were collected after obtaining informed consent from the patients and approval from the Institutional Review Board (no. 4-2009-0180). The study was approved by the ethics committee of Severance Hospital (Yonsei University College of Medicine).

cDNA synthesis of Der f 23 and Der p 23

The house dust mites, *D. farinae* and *D. pteronyssinus*, were obtained from the Arthropods of Medical Importance Resource Bank (AMIB) at the Department of Environmental Medical Biology, Yonsei University College of Medicine (Seoul, Korea). Total RNA was isolated from the frozen mite bodies using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. The cDNA encoding Der f 23 and Der p 23 were amplified by polymerase chain reaction (PCR) using primer sets based on a Der f 23 (GenBank accession Number KM009999.1) and Der p 23 sequence (EU414751.1) (**Figure 1**). The sequences of the used primers were as follows:

Der f 23, 5′-**CTCGAG**AAAAGAGAGGCTGAA GCTGAT ATTGATCATGATGATGATCC-3′(forward) 5′-**GCGGCC** GCTTAATGGTGATGATGGTGATGTGTACATGTTAAT TCTTTTTCATTC-3′ (reverse); Der p 23, 5′-**CTCGAG**AAA AGAGAGGCTGAAGCCGCCAATGATAATGATGATGAT C-3′ (forward) 5′-**GCGGCC**GCTTAATGGTGATGATGG TGATGAGTGCATGTTTCTTCATCTTCA-3′ (reverse).

The underlined sections correspond to Xho I and Not I sites, respectively, and six histidine codons were incorporated in the reverse primer.

Protein expression of recombinant Der f 23 and Der p 23 in Pichia pastoris

Der f 23 and Der p 23 proteins were expressed in *Pichia pastoris* as per the manufacturer's instructions. Briefly, each cDNA was subcloned into pPIC9 vectors (Invitrogen, Waltham, MA, US), and SMD1168 (Invitrogen, Waltham, MA, US) cells were transformed with *Sac* I-linearized plasmids using a *Pichia* EasyComp Kit (Invitrogen, Waltham, MA, US). His+ transformants were selected on Regeneration Dextrose Base agar plates (1.34% yeast nitrogen base without amino acids, 1 M sorbitol, 1% dextrose, 4×10^{-5}% biotin, and 0.005% each of L-glutamic acid, L-methionine, L-lysine, L-leucine, and L-isoleucine). A clone was selected and the cells were grown for 4 days at 220 rpm, with 0.05% methanol (v/v) added every 24 h. The culture supernatant was harvested and concentrated by 80% ammonium sulfate-precipitation. The precipitates were dissolved in 10 mM imidazole, 300 mM NaCl, 50 mM $NaH_2PO_4 \cdot H_2O$ (pH to 8.0). Recombinant protein was purified using nickel nitrilotriacetic acid (NTA) resin (Qiagen, Valencia, CA, USA) in elution buffer (20 mM sodium phosphate, pH 7.4, containing NaCl and 0.5 M imidazole).

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Der f 23    MKFNITIAFV SLAILVHSSY ADIDHDDDPT TMIDVQTTTV QPSDEFECPT 50
Der p 23    .....I.V.I .......... .AN.N..... .TVHP-...E ..D.K....S 49

Der f 23    RFGYFADPKD PCKFYICSNW EAIHKSCPGN TRWNEKELTC T 91
Der p 23    .......... .H........ ..V..D.... .....D.E.. . 90
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Figure 1. Amino acid sequence alignment of Der f 23 and Der p 23. The full-length sequence of Der f 23 (no. AIO08855.1) consists of 91 amino acids with a calculated molecular mass of 10.43 kDa and the full-length sequence of Der p 23 (no. ACB46292) comprises 90 amino acids with a calculated molecular mass of 10.34 kDa.

Table 1. Clinical features of the patients whose serum samples were used in the competitive ELISA.

No.	Age (year)	Sex (M/F)	Clinical symptoms	ELISA results (A450nm)[a]		
				Der f extract	rDer f 23	rDer p 23
1	26	M	Cough	1.848	0.675	0.740
2	20	M	Rhinitis	2.354	2.269	1.805
3	49	F	Asthma	2.710	0.579	0.993
4	36	M	Cough	2.841	0.876	0.752
5	20	M	Asthma	2.032	0.808	2.292
6	63	M	Urticaria, Rhinitis	2.286	0.984	1.747
7	19	F	Atopic Dermatitis	2.803	0.584	0.66
8	29	F	Atopic Dermatitis	2.204	0.974	0.869
9	23	F	Atopic Dermatitis	3.189	1.943	1.737
10	24	M	Rhinitis	2.374	1.291	1.631

[a]The cut-off values were 0.125 for *D. farinae* extract, 0.060 for rDer f 23, and 0.061 for rDer p 23, respectively.

Measurement of IgE reactivity to recombinant Der f 23 and Der p 23

Microtiter plates were coated with 100 μl recombinant protein (2 μg/ml in 50 mM sodium carbonate, pH 9.6) and washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST). The plates were blocked with 1% bovine serum albumin (BSA; EMD Millipore, Kankakee, IL, USA) in PBST for 1 h at room temperature and then incubated for 1 h with 100 μl serum per well diluted at 1:9 in PBST containing 1% BSA. IgE antibodies were detected using biotinylated goat anti-human IgE (Vector Laboratories, Burlingame, CA, USA) and streptavidin-peroxidase (Sigma-Aldrich, St. Louis, MO, USA). The assay was developed with 3,3′,5,5′-tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA), which undergoes a color change in the presence of antibody/allergen complexes. The absorbance at 450 nm was measured using a Tecan sunrise microplate reader (Tecan, Salzburg, Austria) and Magellan CE software after addition of 0.5 M H_2SO_4 to stop color development. The cut-off value was defined as the mean absorbance plus twice the value of the standard deviation of 20 negative controls.

Competitive ELISA

The inhibitory effects of between Der f 23 and Der p 23 was examined using competitive ELISA. The recombinant protein was suspended in a coating buffer (2 μg/ml, 50 mM carbonate buffer, pH 9.6) and added to the wells of ELISA plates. After blocking with 1% BSA in PBST for 1 h at room temperature, wells were incubated with the pooled serum of 10 participants (**Table 1**) who were selected based on their high IgE binding activity to *D. farinae* extract. The pooled serum was pre-incubated overnight at 4°C with solutions containing various concentrations (0.001, 0.01, 0.1, 1.0, or 10.0 μg/ml) of the recombinant proteins. The percentage inhibition was calculated as $(1-A_i/A_0) \times 100$, where A_i stands for absorbance at 450 nm with an inhibitor and A_0 for the absorbance at 450 nm without an inhibitor. These assays were conducted in duplicates.

Statistical Analysis

Correlation coefficients between the IgE reactivity to the two recombinant allergens were analyzed by Pearson's correlation analysis. A p value of < 0.05 was considered to indicate statistical significance.

Results

In this study, recombinant Der f 23 and Der p 23 proteins were expressed in *P. pastoris* and observed as 10 kDa bands in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS PAGE) (**Figure 2**). Subsequently, their IgE-reactivity in Korean patients with allergy who were sensitized to *D. farinae* was assessed. Among the 194 serum samples of HDM-allergic patients that were tested using ELISA, 45.36% (88/194) and 43.81% (85/194) samples showed IgE reactivity to Der f 23 and Der p 23, respectively (**Figure 3**). In addition, 82 house dust mite-sensitized patients reacted to both Der f 23 and Der p 23, while six patients reacted only to Der f 23 and three patients reacted only to Der p 23. There was a strong correlation ($r = 0.877$) between IgE reactivity to rDer f 23 and rDer p 23 (**Figure 4**).

The IgE cross-reactivity between Der f 23 and Der p 23 was assessed using competitive ELISA with pooled serum from 10 patients (**Table 1**). The serum sample preincubated with Der f 23 or Der p 23 did not significantly inhibit IgE reactivity to the *D. farinae* crude extract (**Figure 5A**). However, the serum sample preincubated with 10 μg/ml of rDer p 23 inhibited 86.1% of the IgE reactivity to rDer f 23 (**Figure 5B**), and the serum sample preincubated with 10 μg/ml of rDer f 23 inhibited 61.1% of the IgE reactivity to rDer p 23 (**Figure 5C**).

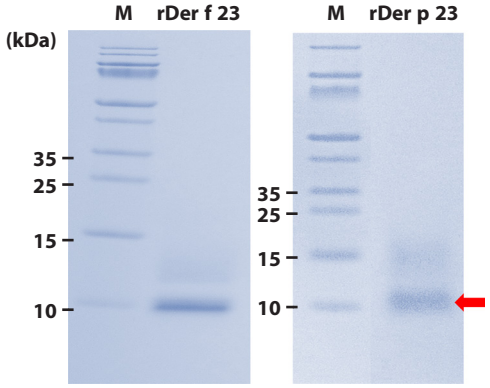

Figure 2. Purification of recombinant allergens Der f 23 and Der p 23. Each recombinant protein (3 μg) was run onto SDS-PAGE gel under reducing conditions.

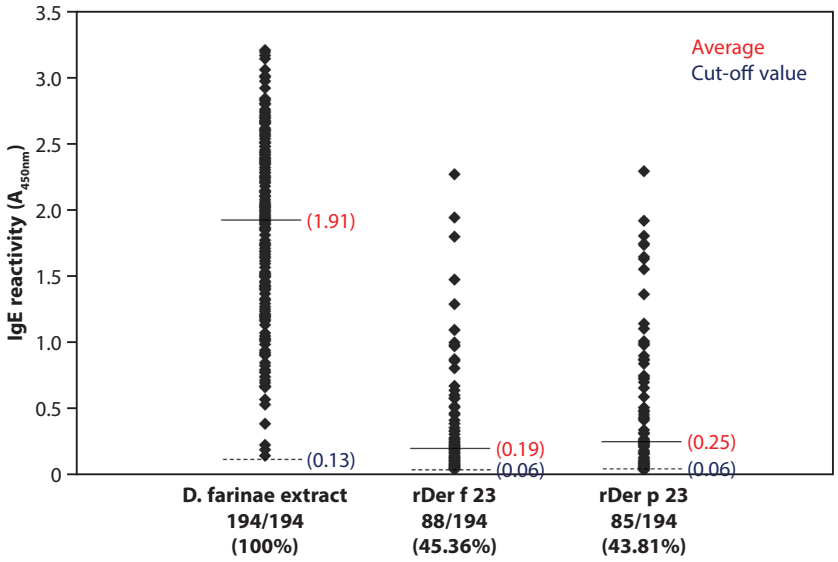

Figure 3. IgE reactivity to recombinant Der f 23 and Der p 23 in sera from allergy patients sensitized with *Dermatophagoides farinae* was tested using ELISA. The cut-off values (dashed lines) were defined as the means plus two standard deviations for the control sera (n = 20).

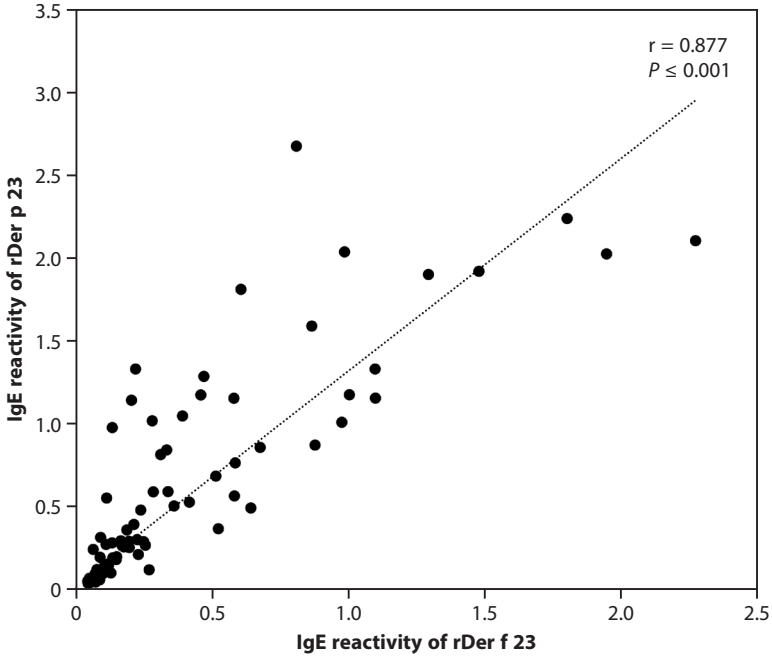

Figure 4. Correlation between the IgE reactivity to rDer f 23 and rDer p 23. "r" represents Pearson's correlation coefficient.

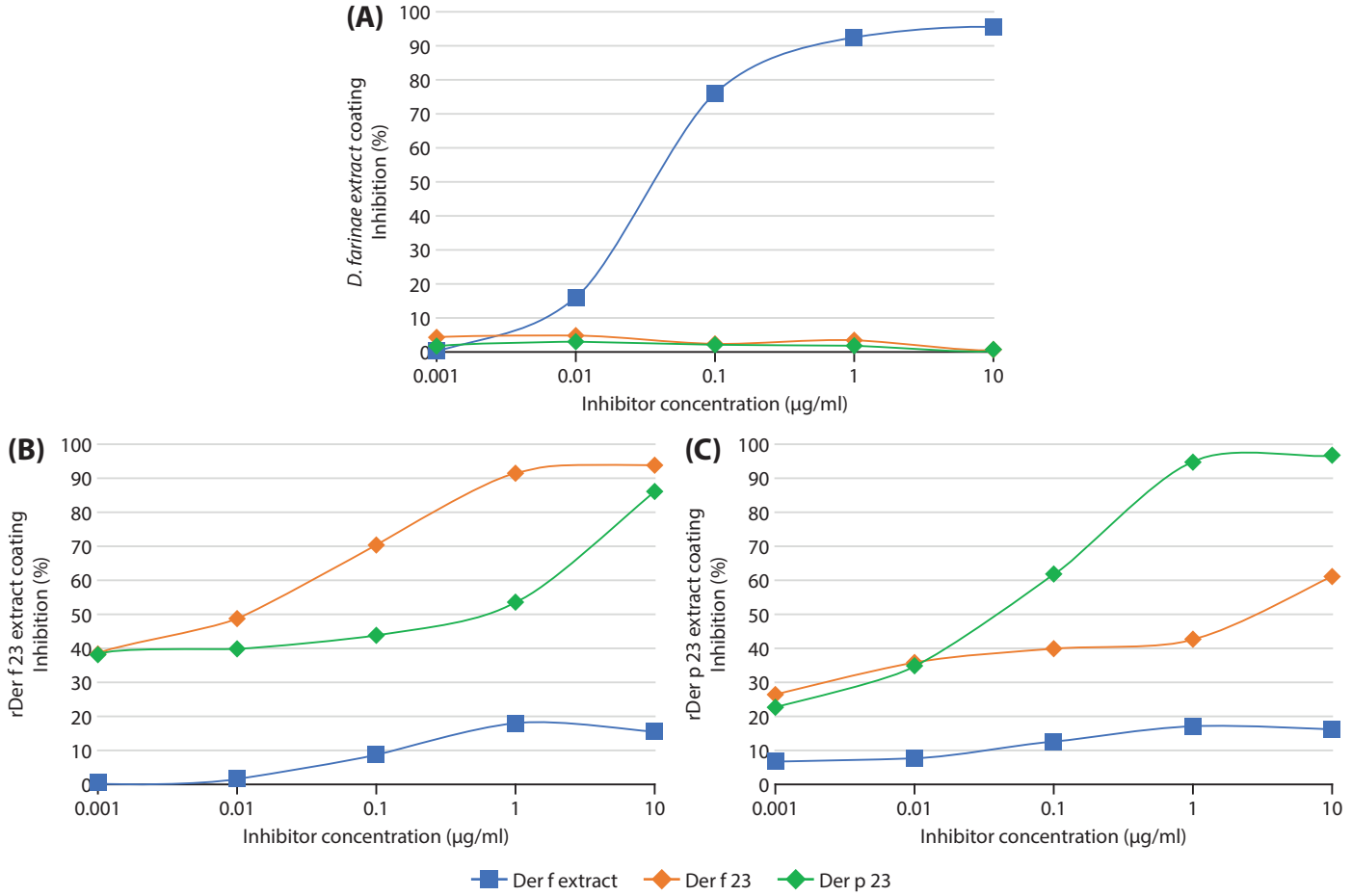

Figure 5. Competitive ELISA analyses of rDer f 23 and rDer p 23. Competitive IgE ELISA was performed using pooled serum from 10 patients, pre-incubated with 0.001, 0.01, 0.1, 1, and 10 μg/ml of each allergen. The ELISA plates were coated with (A) *Dermatophagoides farinae* extract, (B) rDer f 23, and (C) rDer p 23.

Discussion

Group 23 HDM allergens (Der p 23 and Der f 23) have been identified and classified as major allergens.[4,10] It is present only in small quantities in HDM fecal pellets, and despite its homology with chitin-binding proteins, Der p 23 does not interact *in vitro* with chitin matrices.[11] Our study is the first to demonstrate IgE reactivity to HDM group 23 allergens in Korean patients with allergy. According to our results, 45.36% and 43.81% of the serum samples from patients with HDM-allergy showed IgE reactivity to rDer f 23 and rDer p 23, respectively, which are slightly lower rates than those reported in previous studies.

In 2013, Der p 23 was first reported as a major HDM allergen in Austria, and showed IgE reactivity in the sera from 74% of patients with allergy to *D. pteronyssinus*.[5] Furthermore, rDer p 23 induced upregulation of CD203c expression in basophils from patients with *D. pteronyssinus* allergy.[4] A study in Italy reported that 59.8% of serum samples from patients with HDM allergy showed IgE reactivity to Der p 23.[12] Similarly, in Thailand, 54% of HDM allergic patients demonstrated Der p 23-specific IgE responses.[11] More recent studies published between 2020 and 2022 revealed that IgE reactivity rates to Der p 23 were 54.6% in China,[13] 73.5% in Austria,[14] 80% in Italy,[15] 75.0% in Czechia,[16] 55.7% in Ukraine,[17] and 86% in Spain.[6] Der f 23 was recently identified as an allergen in 2019. A study conducted in China reported that Der f 23 was a new major allergen of *D. farinae*, and 55.8% of serum samples from HDM-allergic patients showed specific IgE binding activity to rDer f 23.[10]

Previous studies have reported that IgE reactivity to Der p 23 was related to allergic symptoms and their severity. Asthma was strongly associated with Der p 23 hypersensitivity and asthma severity was associated with Der p 23 IgE levels in an Italian study.[12] In Slovenia, reactivity to Der p 23 was used to distinguish patients with allergic rhinitis from those with asymptomatic HDM sensitization.[7] Another study revealed that Der p 23 mono-sensitized patients had a higher prevalence of rhinitis.[15] The relative risk of atopic dermatitis was found to be increased in patients sensitized to Der p 23.[6] Furthermore, the severity of atopic dermatitis was reported to be significantly associated with sensitization to Der p 23.[16]

In this study, we constructed recombinant Der f 23 and Der p 23 proteins in *Pichia pastoris*, a yeast that supports proper protein folding, post-translational modifications, and glycosylation, which are important for protein stability, similar to the wild type.[17] We found strong IgE cross-reactivity between rDer f 23 and rDer p 23 using competitive ELISA.

The lack of inhibition observed with the crude extract is likely due to the low concentration of Der f 23 in the extract. Since Der f 23 is present in a low concentration in the extract, this could affect both diagnosis and treatment. The IgE cross-reactivity between the two allergens (Der f 23 and Der p 23) could be attributed to their amino acid sequence homology (78%). A previous study using other Der f 23 isoforms reported that Der f 23 showed 71% amino acid sequence homology with Der p 23, which is lower than that found in our study.[10] We have officially registered the allergen Der f 23 in the WHO/IUIS Allergen Nomenclature Database under the designation Der f 23.0201.

This cross-reactivity between allergens can cause confusion while determining the primary sensitizer.[18] In addition, allergic symptoms due to cross-reactivity between allergens may occur, which warrant cautious clinical approach.[19] Component-resolved diagnosis using individual allergen molecules to determine the allergen which has been sensitized can help in the diagnosis and treatment of patients.[20] Furthermore, a clinical approach that considers cross-reactivity can be used for allergen-specific immunotherapy in the future.

A limitation of this study is that we did not perform experiments using native Der f 23; therefore, the actual IgE reactivity might be stronger than what was observed with the recombinant form. In addition, inhibition experiments could not be performed using individual patient serum samples to assess cross-reactivity, due to the insufficient quantity of serum available from each patient.

Conclusion

This study found sera IgE reactivity rates of 45.36% and 43.81% to rDer f 23 and rDer p 23, respectively, and IgE cross-reactivity between these two allergens in HDM-allergic patients in South Korea. This study provides vital information that may be helpful in the development of component-resolved diagnosis and allergen-specific immunotherapy in the future.

Acknowledgements

Not applicable.

Conflict of interest

All authors declare no conflicts of interest regarding the publication of this paper.

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Author contributions

- Myung-hee Yi, PhD collected and analyzed the data, and prepared the manuscript.
- Tai-Soon Yong, MD, PhD designed the study, and reviewed and prepared the manuscript.
- Chung-Ryul Kim, PhD performed the laboratory experiments.
- Kyoung Yong Jeong, PhD critically reviewed the manuscript.
- Ju Yeong Kim, MD, PhD (corresponding author) designed the study, interpreted data, reviewed and prepared the manuscript.

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