Sensitization to Various Minor House Dust Mite Allergens is Greater in Patients with Atopic Dermatitis than in those with Respiratory Allergic Disease

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Directed by Professor Jung-Won Park

The Doctoral Dissertation submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Here, I dedicate this thesis to my precious family.

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ABSTRACT

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Background: Various allergenic proteins are produced by house dust mites (HDM). However, the allergenicity and clinical implications of theses allergens are unknown.

Objective: The purpose of this study was to identify allergens in *Dermatophagoides farinae* and elucidate the sensitization profiles to these in Korean patients suffering from respiratory (allergic rhinitis and/or asthma) and atopic dermatitis symptoms.

Methods: IgE reactivities in sera from 160 HDM allergy patients were analyzed by one- and two-dimensional gel electrophoresis and immunoblotting. IgE-reactive components were identified by liquid chromatography-coupled electrospray ionization–tandem mass spectrometry. Nine recombinant mite allergens (Der f 1, Der f 2, Der f 10, Der f 11, Der f 13, Der f 14, Der f 30, Der f 32, and Der f Alt a 10) were produced, and the IgE reactivity in sera to each was determined by ELISAs.

Results: Der f 1 and Der f 2 were recognized by IgE in serum samples from 88.1% and 78.1% of all patients, respectively. Patients with respiratory allergies were mainly sensitized to these major allergens, whereas patients with atopic dermatitis symptoms showed poly-sensitization to major and minor allergen components (including Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10).

Conclusions: Patients with respiratory allergic disease sensitize to major allergen components of HDM. Those with atopic dermatitis were sensitized to a broader range of minor allergen components of HDM (Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10).

Key words : allergic rhinitis; asthma; atopic dermatitis; house dust mite; recombinant allergen

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I. INTRODUCTION

House dust mites (HDM) are one of the most common sources of indoor aeroallergens in many countries. Due to the chronic perennial exposure, sensitization to these aeroallergens can begin at a young age and develop throughout an individual's lifetime into multiple diseases, including allergic rhinitis (AR), allergic asthma (AA), and atopic dermatitis (AD). Indeed, nearly 50% of asthmatic patients are known to be allergic to HDM.[1]

The main pathogenic species of mites in allergic diseases are *Dermatophagoides farinae* and *D. pteronyssinus*, which account for 90% of HDM allergies.[2] The sensitization profiles to these HDM vary geographically, as *D. farinae* is present in drier areas[3] and *D. pteronyssinus* predominates in humid areas.[3,4]

Various components of HDM (e.g., mite bodies, fecal pellets, egg cases, and

skin casts) are associated with allergic disease.[5] Currently, approximately 28 and 19 allergens have been identified for *D. farinae* and *D. pteronyssinus*, respectively.[5] Each protein has various immunobiological characteristics, resulting in various sensitization patterns and diseases via an allergic march process. The identification of these sensitization patterns is important because it enables accurate diagnosis and treatment, especially with allergen-specific immunotherapy (AIT), which has been shown to be effective for HDM allergies.[6,7] A component-resolved diagnosis discriminates cross-reactivity with other allergens from genuine sensitization and can predict the effectiveness of immunotherapy.[8,9] Indeed, recent studies have shown that immunotherapy is effective for patients with AD caused by HDM[10-13] as well as for those with AA and AR.[14] However, further study and clarification of sensitization profiles are still needed. Therefore, we produced various recombinant HDM allergens and analyzed IgE reactivity profiles from sera of Korean HDM allergy patients with various clinical manifestations.

II. MATERIALS AND METHODS

Subjects

We enrolled HDM allergic patients being treated by specialized allergists and dermatologists at the Allergy and Asthma Center at Severance Hospital in Seoul, Republic of Korea, from 2015 to 2016. This tertiary teaching hospital has more than 2,400 beds and treats patients from all over the Republic of Korea. The

criteria for study inclusion were patients aged 6–80 years, those who were diagnosed with HDM allergic diseases (AA, AR, or AD) by an allergy specialist, and those with HDM sensitization determined by a skin prick test or the detection of IgE specific to *D. farinae* (hereafter designated sIgE).

Asthma was diagnosed based on GINA guidelines,[15] with typical symptoms and signs, including wheezing, shortness of breath, chest tightness or cough, variable airway obstruction confirmed with spirometers, presence of the bronchodilator response, and bronchial provocation tests. Induced sputum and fractional exhaled nitric oxide was also checked for supportive information. Diagnosis of allergic rhinitis was based on ARIA guidelines,[16] with symptom and sings of rhinorrhea, nasal obstruction, and itching sensation or sneezing, and meaningful sensitization to HDM. We determined whether the symptoms of allergic diseases were exacerbated after mite exposure (e.g., after making the bed, cleaning a closet, or sleeping on old bedding). Atopic dermatitis was diagnosed when atopy was accompanied by chronic relapsing eczematous dermatitis with pruritis.[17]

HDM-sensitized patients receiving AIT were excluded. The patients were divided into three groups for comparative analysis: (i) patients with airway allergic disease (AA or AR), (ii) patients with both airway allergic disease and AD (AA/AR+AD), and (iii) patients with AD only. Blood samples were collected from each study participant and stored at -70°C. The Institutional Review Board of the Yonsei University Health System approved this study (no.

4-2013-0397). All participants provided written informed consent prior to participating in the study.

Skin prick test

Skin prick tests were performed using 53 common inhalant allergens (including samples of HDM, tree, grass, and weed pollens, molds, animal dander, and cockroaches). The negative control was normal saline with 0.3% phenol and 50% glycerol, and 0.1% histamine (Allergy Therapeutics, Worthing, UK) was used as a positive control. Results were interpreted after 15 minutes: wheal sizes greater than 3 mm were considered as positive reactions. All participants were instructed to discontinue medications that might influence the test results.

sIgE determination by ImmunoCAP

To detect sIgE in serum samples, we used the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden), which has a detection range for sIgE of 0.1 kU_A/L to 100 kU_A/L. sIgE titers greater than 0.35 kU_A/L were designated positive. In addition to total extract allergen (*D. farinae*) sIgE, the commercially available mite component allergens Der p 1, Der p 2, and Der p 10 sIgEs were also measured.

SDS-PAGE and immunoblotting

Standardized *D. farinae* extracts were kindly provided by the Yonsei Allergy

Institute (Seoul, Republic of Korea). Freeze-dried mite bodies were defatted and extracted as described previously.[4] The extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 15% gels and transferred to polyvinylidine difluoride (PVDF) membranes (0.45-μm pore; GE Water & Process Technologies, Trevose, PA, USA). The membranes were incubated in 3% skim milk overnight, and then incubated in patients' serum samples overnight at 37°C. Subsequently, the membranes were incubated for 1 h in diluted (1:1,000) alkaline phosphate-conjugated goat anti-human IgE (Sigma-Aldrich). Nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI) were used for color development.

Two-dimensional gel electrophoresis and mass spectrometry

HDM extracts were prepared by desalting with trichloroacetic acid as described previously,[4] and then 0.1 mg samples were run on isoelectric focusing (pH 3–10) gels and separated on 15% SDS-polyacrylamide gels. The separated proteins were stained with Coomassie blue or transferred onto PVDF membranes for immunoblotting. Subsequently, IgE-reactive components were probed overnight with sera pooled from 20 subjects (serum diluted 1:4 in phosphate-buffered saline). IgE antibodies were then detected using 1:1,000 diluted alkaline phosphate-conjugated goat anti-human IgE (Sigma-Aldrich) and visualized with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate. To identify the proteins reactive with IgE antibodies, liquid

chromatography-coupled electrospray ionization–tandem mass spectrometry analysis was carried out at ProteomeTech (Seoul, Republic of Korea). The amino acid sequences of the identified HDM-associated proteins were used to generate HDM component allergens.

HDM component allergens

Recombinant Der f 1, Der f 2, and Der f 10 were produced as described previously.[18] Briefly, a mutant pro-form of Der f 1 with a C-terminal histidine tag was produced in the *Pichia* expression system. Der f 2 from inclusion bodies and Der f 10 from soluble fractions were expressed in *Escherichia coli* and purified using Ni-nitrilotriacetic acid resin (Qiagen, Valencia, CA). Peptide fragments for Der f 11, which are known to be immunodominant, were expressed in E. coli.[19] For Der f 14, partial C-terminal sequences were produced, as protease-degraded peptide fragments have been shown to have greater allergenicity.[20]

For the cloning of Der f 13, Der f 30, Der f 32, Der f Alt a 10, and Der f 11 and Der f 14 peptide fragments, first strand cDNAs were synthesized using total RNA from the HDM (Yonsei Allergy Institute, Seoul, Republic of Korea). The coding regions of each allergen were amplified by PCR using oligonucleotide primers designed using sequences obtained from GenBank (Table 1). cDNA fragments of each allergen were ligated into the pEXP5NT TOPO vector (Invitrogen, Carlsbad, CA) and transformed into *E. coli* BL21(DE3). The

expression of recombinant allergens was induced by 1 mM isopropyl-1-thio-β-D-galactopyranoside, and the allergens were purified using Ni-nitrilotriacetic acid resin. All recombinant allergens except Der f 11 peptide fragments were isolated from inclusion bodies and separated on SDS-polyacrylamide gels under reducing conditions, and protein concentrations were determined by a Bradford assay.

Table 1. Primers used for cloning recombinant allergens

Allergen	Host cell	Primer	Sequence (5′→3′)
rDer f 1	*Pichia*	Forward	CTCGAGCGTCCAGCTTCAATCAAAACT
		Reverse	GGCCGCTTAGTGATGGTGATGGTGATG
			CGCGCCGCGTGATGGTG
rDer f 2	*E. coli*	Forward	GATCAAGTCGATGTTAAAG
		Reverse	TCAAACAATGTTTTTTGT
rDer f 10	*E. coli*	Forward	ATGGAGGCCATCAAGAAA
		Reverse	CTGTCTGCGTAATATGAAAG
rDer f 11*	*E. coli*	Forward	CACATTGAATCGGAAGAAACG
		Reverse	TGATTCTAATTCCACTTCCAA
	E. coli	Forward	ATGGCAAGCATTGAAGGTAA
		Reverse	TTAGATTCGTTTATATGTTC
rDer f 13	*E. coli*	Forward	ATGGATCCGTCAACATTGAG
		Reverse	TCAGTTGTCTTCGACGATGAAATT
rDer f 14*	*E. coli*	Forward	ATGGCTGCTAATCCTGAATC
		Reverse	TTACGATGAATGCAATGTATGAC
rDer f 30	*E. coli*	Forward	ATGTCTACTACAAATTATTC
		Reverse	TTAGATCAATTTAACATGATGCC
rDer f 32	*Pichia*	Forward	ATGGCCCAAGTGGAAGTAAAA
		Reverse	CTAATTTAAATTCGAATTTTTATTC
rDer f Alt a 10	*E. coli*	Forward	ATGGCCCAAGTGGAAGTAAAATATAC
		Reverse	CTAATTTAAATTCgAATTTTTATTC

*Peptide fragment.

ELISAs

For enzyme-linked immunosorbent assays (ELISAs), recombinant proteins (2 μg/mL) were coated on microplates in 0.05 M carbonate buffer (pH 9.6) and kept at 4°C overnight. After washing with PBST, the plates were blocked with 3% skim milk in PBST. Serum samples diluted 1:4 in PBST containing 1% bovine serum albumin were dispensed into the wells of the microplate and incubated for 1 h. Subsequently, IgE antibodies were detected by adding 1:1,000 diluted biotinylated goat anti-human IgE (Vector, Burlingame, CA) and 1:1,000 diluted streptavidin peroxidase (Sigma-Aldrich). Color development was conducted by 3,3′,5,5′- tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD). After the addition of 0.5 M H_2SO_4, the absorbance was measured at 450 nm. The cutoff value was determined as the mean absorbance plus three times the standard deviation obtained from the non-allergic negative controls.

Statistical analysis

The results were analyzed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp Armonk, NY). Nonparametric continuous data were analyzed by Kruskal–Wallis tests and categorical data were analyzed by Fisher's exact or Pearson's chi-squared tests. For multiple comparisons between the groups, a Dunn's test was performed after the Kruskal–Wallis test. A P value of < 0.05 was considered significant.

III. RESULTS

Characteristics of study population

The demographic data of the 160 HDM allergic patients that participated in the study are shown in Table 2. Wheal sizes against *D. farinae* did not differ among the groups divided by clinical diagnosis. However, the patients diagnosed with AD were younger ($P < 0.001$) and had higher total IgE and sIgE titers ($P < 0.001$). Allergic conjunctivitis and food and drug allergies were more prevalent in patients with airway disease than in those with AD only.

Table 2. Baseline characteristics of enrolled patients

Characteristic	Total (n = 160)	AA/AR (n = 67)	AA/AR+AD (n = 41)	AD (n = 52)	P value*
Age (years)	26.7 ± 15.7	33.6 ± 17.9	21.1 ± 10.9	22.2 ± 12.4	<0.001
Male : Female (%)	60.6 : 39.4	56.7 : 43.3	65.9 : 34.1	61.5 : 38.5	0.573
Diagnosis, *n* (%)					
AA	68 (42.5)	49 (72.1)	19 (46.3)	0 (0)	<0.001
AR	102 (63.8)	65 (97.0)	37 (90.2)	0 (0)	<0.001
AD	93 (58.1)	0 (0)	41 (100)	52 (100)	<0.001
Allergic conjunctivitis	33 (20.6)	17 (25.4)	14 (34.1)	2 (3.8)	0.001
Food allergy	18 (11.3)	14 (20.9)	1 (2.4)	3 (5.8)	0.002
Drug allergy	4 (2.5)	4 (6.0)	0 (0)	0 (0)	0.031
Total IgE (kU/L)	1,514± 1,637	548 ± 585	1,911±1,859	2,147±1,747	<0.001
D. farinae sensitivity					
Wheal size (mm)	8.4 ± 5.7	8.5 ± 6.0	8.0 ± 5.5	8.2 ± 3.8	0.875
sIgE level (kU_A/L)	53.6 ± 41.1	31.8 ± 29.9	64.3 ± 43.1	73.2 ± 39.2	<0.001

Values are means ± standard deviations, unless otherwise noted. AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis. $^{*}P$ value obtained by statistical analysis comparing 3 groups (AA/AR, AA/AR+AD and AD)

IgE reactivity

The protein profile of *D. farinae* separated by 15% SDS-PAGE under a denaturing condition is depicted in Figure 1A. Sera pooled from subjects with respiratory allergies showed IgE reactivity, with a single prominent band of 14 kDa in size (Figure 1B). By contrast, patients with AD had IgE reactivities to multiple mite proteins (Figure 1C and D). For each disease group, sera from 20 representative patients were pooled. All the immunoblot experiments were performed twice, and the results were consistent (data not shown).

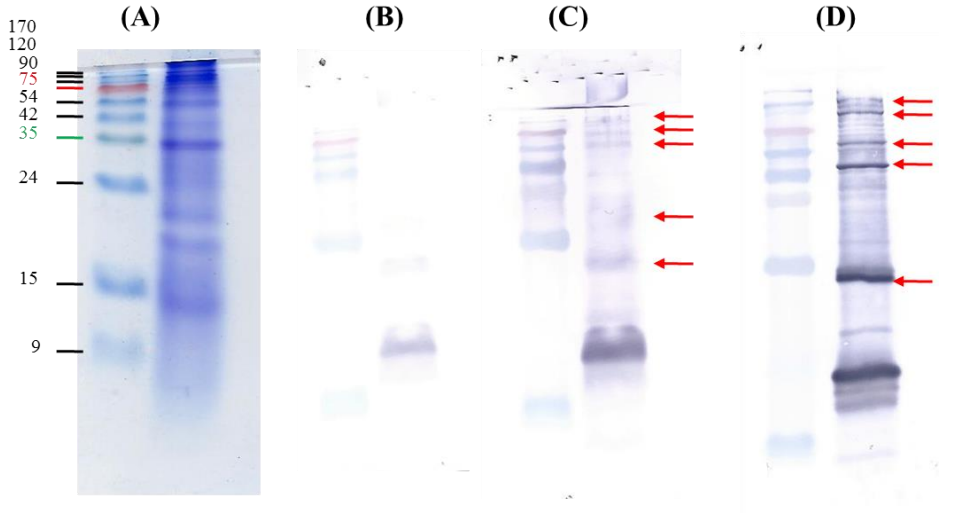


Figure 1. Differences in IgE reactivity between respiratory allergy and atopic dermatitis patients. (A) Protein profile of *Dermatophagoides farinae*. (B) IgE reactivities in samples from patients with respiratory allergy (n=20), (C) respiratory allergy with atopic dermatitis (n=20), and (D) atopic dermatitis only (n=20).

These data were validated by results from two-dimensional electrophoresis followed by Western blotting (Figure 2). Consistent with the SDS-PAGE results, a single 14-kDa protein was detected in pooled sera from patients (n=20) with AA or AR (Figure 2B), whereas multiple bands were observed from patients with AD (Figure 2C and D).

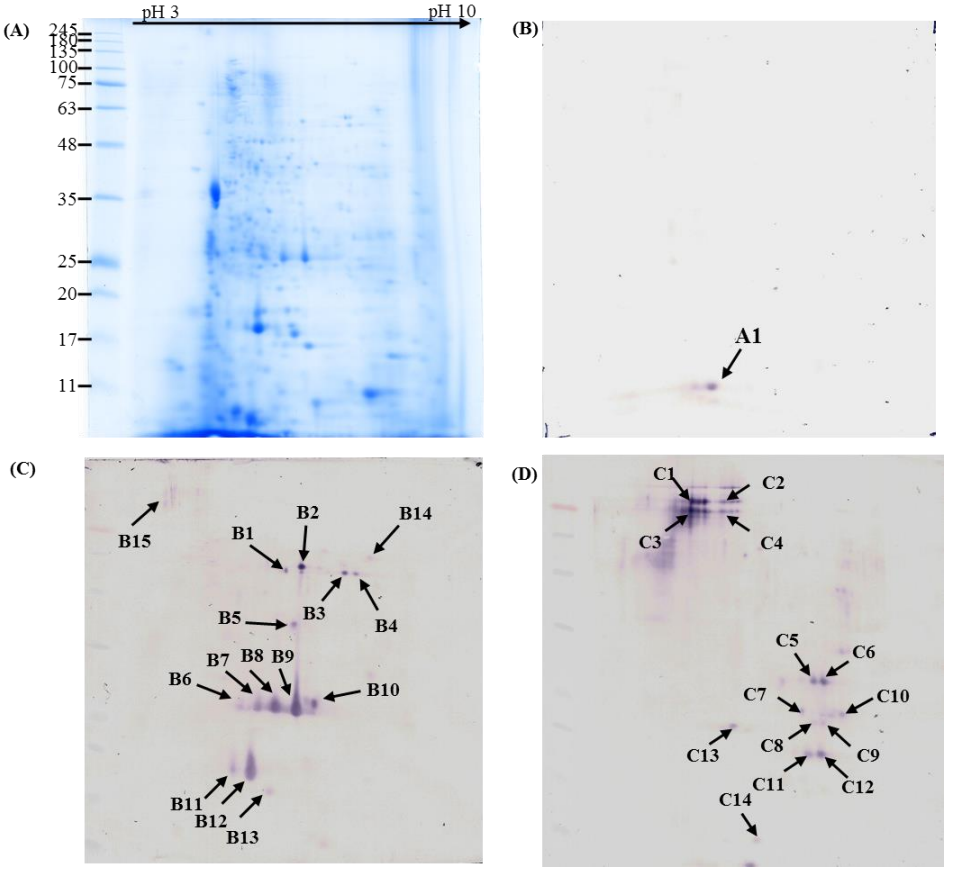


Figure 2**.** Differences in IgE reactivity between respiratory allergy and atopic dermatitis patients analyzed by two-dimensional protein separation. (A) Protein profile of *Dermatophagoides farinae*. IgE reactivities in pooled sera from patients with (B) respiratory allergy, (C) respiratory allergy with atopic dermatitis, and (D) atopic dermatitis. Arrows indicate spots analyzed further by liquid chromatography-coupled electrospray ionization–tandem mass spectrometry.

The spots denoted with arrows were analyzed by liquid chromatography-coupled electrospray ionization–tandem mass spectrometry (Table 3). The 14-kDa spot (A1) in the AA/AR group was identified as Der f 2. Among the 14 spots (B1–B14) observed in samples from patients with AA/AR and AD and 13 spots (C1–C13) from patients with AD only, Der f 1, Der f 2, Der f 11, Der f 13, Der f 14, Der f 30, and Der f Alt a 10 were identified.

Table 3. Proteins identified from two-dimensional Western blot

Spot	Identification (organism)	Mass (Da)	Score
A1	Der f 2	14,026	333
	Der f 13	14,971	103
B1	Nesprin-1 (*Cerapachys biroi*)	129,899	66
B2	Der f 1	23,763	100
B4	Nesprin-1 (*Cerapachys biroi*)	129,899	59
B6	Der f 1	23,763	209
	Der f 32	33,951	70
B7	Der f 1	23,763	145
B8	Der f 1	23,763	208
B9	Der f 1	23,763	179
B10	Der f Alt a 10 allergen (unpublished)	54,162	83
B11	Der f 30	19,770	169
B12	Der f 30	19,770	248
B13	Der f 30	19,770	137
B14	AGAP009853-PA-like protein (*Anopheles sinensis*)	46,460	58
C1	Der p 14	190,542	97
C2	Der f 11	102,407	510
C3	Mag 3 allergen	40,520	79
C4	Der f 11	102,407	1,055
C6	Der f 14	39,643	295
C9	Alpha-enolase	47,214	62
C10	Not detected		
C11	Der f 14	39,643	65
C12	Der f 14	39,643	93
C13	Cytochrome P450 3A9 (*Crassostrea gigas*)	101,332	60

Sensitization profiles for recombinant *D. farinae* component allergens

To further examine IgE reactivities to the identified allergens, recombinant allergens were produced (Figure 3 and Table 4) for use in ELISAs.

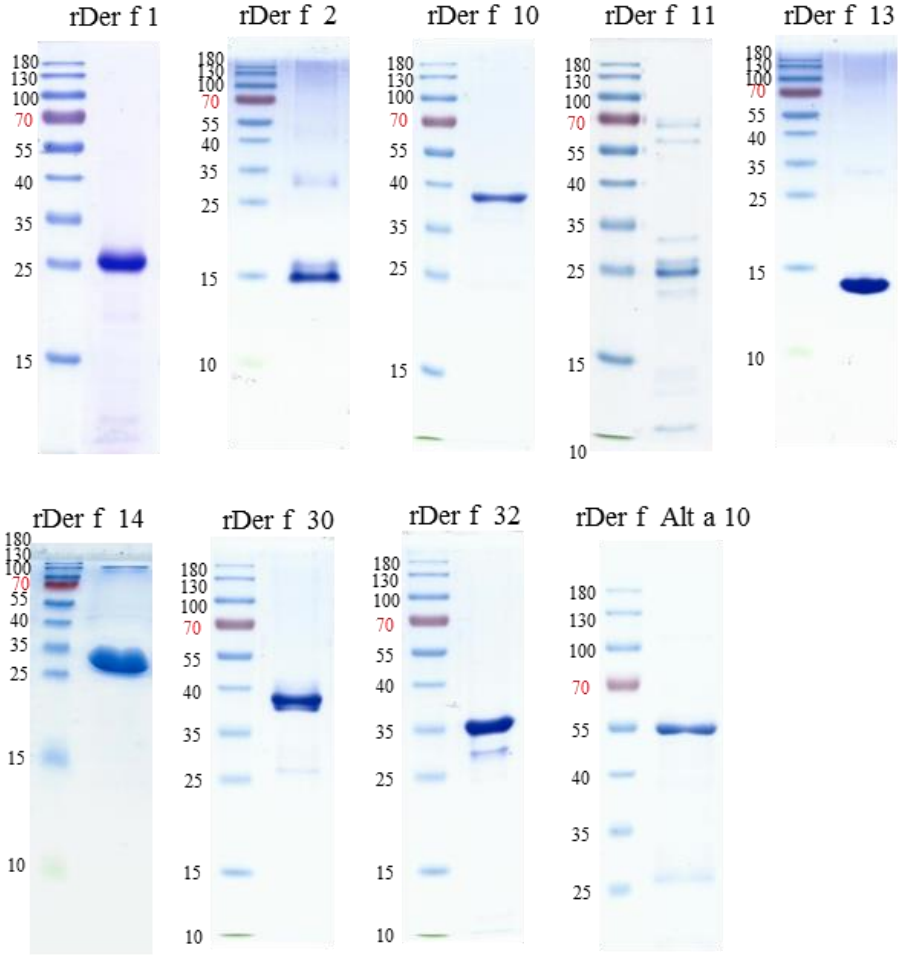


Figure 3. SDS-PAGE of recombinant *Dermatophagoides farina* allergens.

Table 4. Biochemical properties and amino acid sequence of house dust mite component allergens

Allergen (Biochemical name)	N/R	Amino Acid Sequence	Molecular mass (kDa)
Der f 1 (Cysteine protease)	N	TSACRINSVNVPSELDLRSLRTVTPIRMQGGCGSCWAFSGVAATESAYLAYRQTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQQNGVVEERSYPYVAREQQCRRPNSQHYGISNYCQIYPPDVKQIREALTQTHTAIAVIIGIKDLRAFQHYDGRTIIQHDNGYQPNYHAVNIVGYGSTQGVDYWIVRNSWDTTWYDSGYGYFQAGNNLMMIEQYPYVVIM	25.2
	R	TSACRINSVNVPSELDLRSLRTVTPIRMQGGCGSCWAFSGVAATESAYLAYRQTSLDLSEQELVDCASQHGCHGDTIPRGIEYIQQNGVVEERSYPYVAREQQCRRPNSQHYGISNYCQIYPPDVKQIREALTQTHTAIAVIIGIKDLRAFQHYDGRTIIQHDNGYQPNYHAVNIVGYGSTQGVDYWIVRNSWDTTWYDSGYGYFQAGNNLMMHHHHHH	24.8
Der f 2 (NPC2 family)	N	DQVDVKDCANNEIKKVMVDGCHGSDPCIIHRGKPFTLEALFDANQNTKTAKIEIKASLDGLEIDVPGIDTNACHFMKCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKLIGDNGVLACAIATHGKIRD	15.7
	R	MSGSHHHHHHGSSGENLYFQSLDQVDVKDCANNEIKKVMVDGCHGSDPCIIHRGKPFTLEALFDANQNTKTAKIEIKASLDGLEIDVPGIDTNACHFMKCPLVKGQQYDIKYTWNVPKIAPKSENVVVTVKLIGDNGVLACAIATHGKIRD	16.5
Der f 10 (Tropomyosin)	N	MEAIKKKMQAMKLEKDNAIDRAEIAEQKARDANLRAEKSEEEVRALQKKIQQIENELDQVQEQLSAANTKLEEKEKALQTAEGDVAALNRRIQLIEEDLERSEERLKIATAKLEEASQSADESERMRKMLEHRSITDEERMDGLENQLKEARMMAEDADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVVGNNLKSLEVSEEKAQQREEAYEQQIRIMTAKLKEAEARAEFAERSVQKLQKEVDRLEDELVHEKEKYKSISDELDQTFAELTGY	32.9
	R	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRDPNMEAIKKKMQAMKLEKDNAIDRAEIAEQKARDANLRAEKSEEEVRALQKKIQQIENELDQVQEQLSAANTKLEEKEKALQTAEGDVAALNRRIQLIEEDLERSEERLKIATAKLEEASQSADESERMRKMLEHRSITDEERMDGLENQLKEARMMAEDADRKYDEVARKLAMVEADLERAEERAETGESKIVELEEELRVVGNNLKSLEVSEEKAQQREEAYEQQIRIMTAKLKEAEARAEFAERSVQKLQKEVDRLEDELVHEKEKYKSISDELDQTFAELTGYSSSVDKLAAALEHHHHHH	38.6
Der f 11* (Paramyosin)	N	MSARTAKYMYRSSGAGASGDISVEYGTDLGALTRLEDKIRLLSDDLESEREMRQRIEREKAELQIQVMSLGERLEEAEGSSESVTEMNKKRDSELAKLRKLLEDVHIESEETAHHLRQKHQAAI	102.4

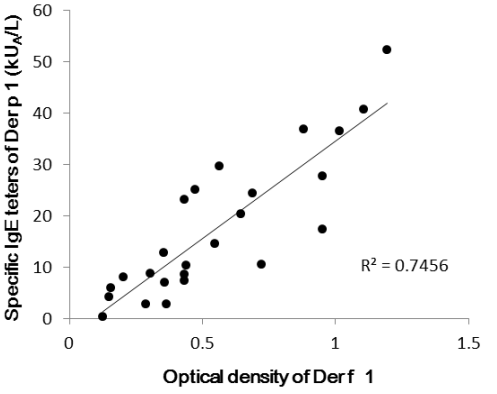
		QEMQDQLDQLQKAKNKSDKEKQKFQAEVFELLAQLETANK EKLTALKNVEKLEYTVHELNIKIEEINRTVIELTSHKQRLSQE NTELIKEVHEVKLQLDNANHLKTQIAQQLEDTRHRLEEEER KRASLENHAHTLEVELESLKVQLDEESEARLELERQLTKAN GDAASWKSKYEAELQAHADEVEELRRKMAQKISEYEEQLE ALLNKCSSLEKQKSRLQSEVEVLIMDLEKATAHAQQLEKRV AQLEKINLDLKNKLEEVTMLMEQAQKELRVKIAELQKLQHE YEKLRDQRDQLARENKKLTDDLAEAKSQLNDAHRRIHEQEI EIKRLENERDELSAAYKEAETLRKQEEAKNQRLIAELAQVR HDYEKRLAQKDEEIEALRKQYQIEIEQLNMRLAEAEAKLKT EIARLKKKYQAQITELELSLDAANKANIDLQKTIKKQALQIT AELQAHYDEVHRQLQQAVDQLGVTQRRCQALQAELEEMRI ALEQANRAKRQAEQLHEEAVVRVNELTTINVNLASAKSKLE SEFSALQADYDEVHKELRISDERVQKLTIELKSTKDLLIEEQE RLVKLETVKKSLEQEVRTLHVRIEEVEANALAGGKRVIAKL ESRIRDVEIEVEEERRRHAETDKMLRKKDHRVKELLLQNEE DHKQIQLLQEMTDKLNEKVKVYKRQMQEQEGMSQQNLTR VRRFQRELEAAEDRADQAESNLSFIRAKHRSWVTTSQVPGG TRQVFTTQEETTNY	
	R	MSGSHHHHHHGSSGENLYFQSLHIESEETAHHLRQKHQAAI QEMQDQLDQLQKAKNKSDKEKQKFQAEVFELLAQLETANK EKLTALKNVEKLEYTVHELNIKIEEINRTVIELTSHKQRLSQE NTELIKEVHEVKLQLDNANHLKTQIAQQLEDTRHRLEEEER KRASLENHAHTLEVELES	21.4
Der f 13 (Fatty acid binding protein)	N	MASIEGKYKLEKSEKFDEFLDKLGVGFMVKTAAKTLKPTFE VAIENDQYIFRSLSTFKNTEAKFKLGEEFEEDRADGKRVKTV IQKEGDNKFVQTQFGDKEVKIIREFNGDEVVVTASCDGVTS VRTYKRI	14.9
	R	MSGSHHHHHHGSSGENLYFQSLMASIEGKYKLEKSEKFDEF LDKLGVGFMVKTAAKTLKPTFEVAIENDQYIFRSLSTFKNTE AKFKLGEEFEEDRADGKRVKTVIQKEGDNKFVQTQFGDKE VKIIREFNGDEVVVTASCDGVTSVRTYKRI	17.4
Der f 14* (Apolipophorin)	N	VTALELLLKGETEDKTRRYVAELTAVGSPSNKQAKAQIEVT KGEEYKITLKSPEHEFNTEFTIHADKNNLKMHMDFPNVFQA DLTGTFQHDKENNVRKNQLNLQYKFAGDEKPHTVDYENEF SFNLKRSSKDKNSGVDYRAKYMSSHFPILNHKVNIQFKYRPF KVNELNLEGEFGRELQHKFQLMRNSQIEVEEVRPFKMHGNS DIKLMANDLDIDYDLKSEFKYESNKGTPIELQYKISGKDRSK RAADLGAEDVEGVIDYKNNGSPIDSKMHAHLKMKGNNYG YDSELKQTQPQQYEGKITLSKNDKKIFINHKSEMTKPTNTFH LKTDADVSYSDSDMKKHYQME	40.5
	R	MSGSHHHHHHGSSGENLYFQSLMDPSTLSLVTKADGKIDMT VDLISPVTKRASLKIDSKKYNLFHEGELSASIVNPRLSWHQY TKRDSREYKSDVELSLRSSDIALKITMPDYNSKIHYSRQGDQI NMDIDGTLIEGHAQGTIREGKIHIKGRQTDFEIESNYRYEDG KLIIEPVKSENGKLEGVLSRKVPSHLTLETPRVKMNMKYDR YAPVKVFKLDYDGIHFEKHTDIEYEPGVRYKIIGNGKLKDD GRHYSIDVQGIPRKAFNLDADLMDFKLKVSKPEDSNKAQFS	38.3

		YTFNEYTETEEYEFDPHRAYYVNWLSSIRKYIQNFIVEDN	
Der f 30 (Ferritin)	N	MAANPESTTKTSRVRMNIQINLEFYASYVYQQMAYHFNRD DVALPGFEKFFDVSSKEEREHAERFMKLQNQRGGRIVLDDI HKPQQQDWSSGLEAMRAALELEKTVNQALLDLHAVATKH NDAQFADFIETHYLTEQVEAIKKLADYITNLERCGPGLGEYL FDRHTLHSS	19.7
	R	SGSHHHHHHGSSGENLYFQSLMAANPESTTKTSRVRMNIQI NLEFYASYVYQQMAYHFNRDDVALPGFEKFFDVSSKEERE HAERFMKLQNQRGGRIVLDDIHKPQQQDWSSGLEAMRAAL ELEKTVNQALLDLHAVATKHNDAQFADFIETHYLTEQVEAI KKLADYITNLERCGPGLGEYLFDRHTLHSS	22.2
Der f 32 (Secreted inorganic pyrophosphatase)	N	MSTTNYSVDHRGSFNSLDYRIYFKDNSNGKIISPWHDIPLFV DKSAKHYNMVVEIPRWTNEKMEIATAEPMSPIKQDIKKGAL RYVKNVFPHKGYIWNYGAFPQTWENPNHIDQDTKTKGDND PIDVIEIGSRVAKRGDVVPVKILGTIALIDEGETDWKIIAIDTR DELASQMNNVDDVEKLLPGLLRATVEWFKIYKIPDGKPANK FAFNGEAKDREFAEKIVEETHQYWQEMMENKSGEHKLDLK NVTLGNSFSINDEQAKQFLETRPSSDAVEPTPIADQVAIDKW HHVKLI	33.9
	R	MSGSHHHHHHGSSGENLYFQSLMSTTNYSVDHRGSFNSLD YRIYFKDNSNGKIISPWHDIPLFVDKSAKHYNMVVEIPRWTN EKMEIATAEPMSPIKQDIKKGALRYVKNVFPHKGYIWNYGA FPQTWENPNHIDQDTKTKGDNDPIDVIEIGSRVAKRGDVVPV KILGTIALIDEGETDWKIIAIDTRDELASQMNNVDDVEKLLP GLLRATVEWFKIYKIPDGKPANKFAFNGEAKDREFAEKIVEE THQYWQEMMENKSGEHKLDLKNVTLGNSFSINDEQAKQFL ETRPSSDAVEPTPIADQVAIDKWHHVKLI	36.4
Der f Alt a 10 (Aldehyde dehydrogenase [homologous to fungus allergen Alt a 10])	N	MAQVEVKYTQIFINNEWHDSISGKTFETINPFTEEKLANVQE GDKADIDRAVVAAVDAFRFDSPWRQMDASQRGHLLYRLA DLIERDQDYIASLESMDNGKPKTMALFDVDLAIKVFRYYAG YADKIHGKTIPADGKVFAFTRIEPVGICGQIVPWNFPFLMAS WKFGPALCAGNTVVLKPAEQTPLSALYLASLTKEGGFPPGV VNVVPGFGETAGAALVDNPKVDKIAFTGSTEIGKLIMRNGS HSMKRITLELGGKSPLVVTENVEDIAQAARTAQDSCFLNMG QCCCAGTRTFVHESIYDEFVKHSVEYCQSHVFGNPFDSKTAF GPQVDKIQMNRILEMIESGKQEGARCVAGGNRMDKRGYFV EPTVFADVTDGMRIAREEIFGPVQQILKYKTLDEVIERCNDT NYGLGSAILTNDINEAMKFSRSIRAGSVWINIPYMIPVSVQTP FGGFKESGVGRELGEDGLRGYGEIKTVVIMDREKKM	54.1

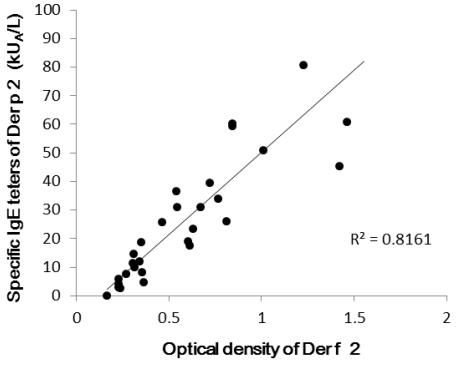
R	MSGSHHHHHHGSSGENLYFQSLMAQVEVKYTQIFINNEWH DSISGKTFETINPFTEEKLANVQEGDKADIDRAVVAAVDAFR FDSPWRQMDASQRGHLLYRLADLIERDQDYIASLESMDNG KPKTMALFDVDLAIKVFRYYAGYADKIHGKTIPADGKVFAF TRIEPVGICGQIVPWNFPFLMASWKFGPALCAGNTVVLKPAE QTPLSALYLASLTKEGGFPPGVVNVVPGFGETAGAALVDNP KVDKIAFTGSTEIGKLIMRNGSHSMKRITLELGGKSPLVVTE NVEDIAQAARTAQDSCFLNMGQCCCAGTRTFVHESIYDEFV KHSVEYCQSHVFGNPFDSKTAFGPQVDKIQMNRILEMIESGK QEGARCVAGGNRMDKRGYFVEPTVFADVTDGMRIAREEIF GPVQQILKYKTLDEVIERCNDTNYGLGSAILTNDINEAMKFS RSIRAGSVWINIPYMIPVSVQTPFGGFKESGVGRELGEDGLR GYGEIKTVVIMDREKKM	56.6

To validate this method, we compared the results from the ELISAs (Der f 1, Der f 1 and Der f 10) with the sIgE titers from the ImmunoCAP assay (Der p 1, Der p 2 and Der p 10), which cannot be used for recombinant component allergens of *D. farinae*, and found that they were indeed correlated (R^2 = 0.75 - 0.82: see Figure 4).

(A) Group 1 allergen



(B) Group 2 allergen



(C) Group 10 allergen

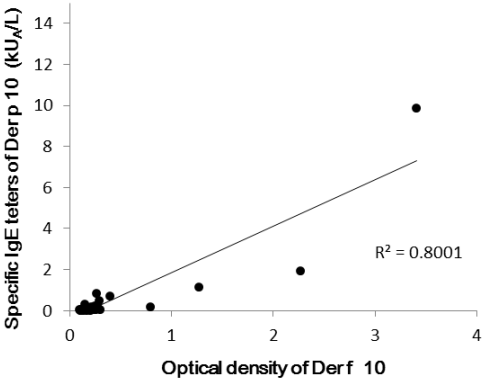
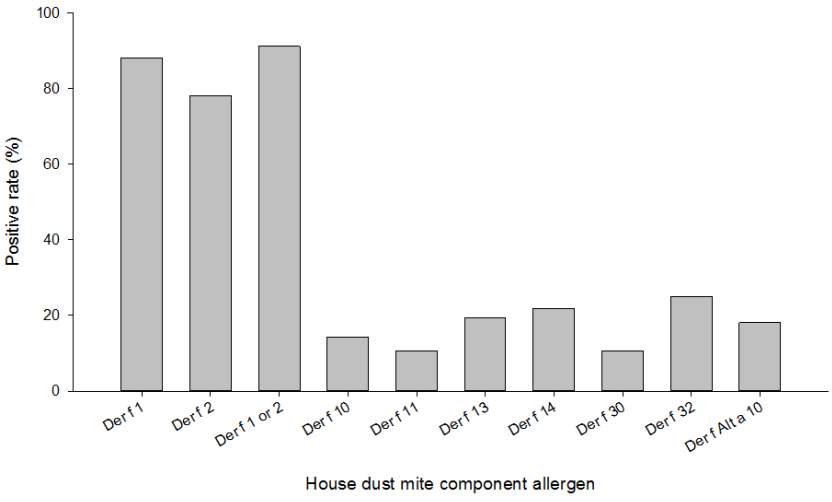


Figure 4. IgE reactivity comparisons of house dust mite component allergens by ELISA and ImmunoCAP

Overall, 91% of the patients were found to be sensitized to Der f 1 or Der f 2 major allergens (Figure 5A; see also Table 5). Fourteen patients (8.8%) were sensitized only to minor allergens. The sensitization profiles for each group are shown in Figure 5B. Sensitization rates to Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10 were higher in the AD group than in the AA/AR group (Table

5).

(A)



(B)

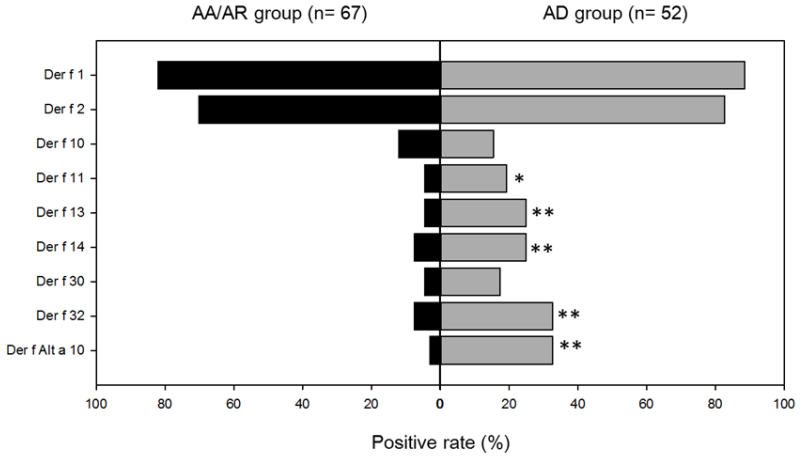


Figure 5. Sensitization profiles of house dust mite component allergens by disease. Profiles from (A) all allergic patients and (B) those with airway or cutaneous allergies. AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis. *$P < .05$, ** $P < .01$

Table 5. Sensitization profiles of component allergens (*n* [%])

Allergen	Total (*n* = 160)	AA/AR (*n* = 67)	AA/AR+AD (*n* = 41)	AD (*n* = 52)	*P* value
Der f 1	141 (88.1)	55 (82.1)	40 (97.6)	46 (88.5)	0.054
Der f 2	125 (78.1)	47 (70.1)	35 (85.4)	43 (82.7)	0.112
Der f 1 or 2	146 (91.3)	57 (85.1)	40 (97.6)	49 (94.2)	0.070
Der f 10	23 (14.4)	8 (11.9)	7 (17.1)	8 (15.4)	0.738
Der f 11	17 (10.6)	3 (4.5)	4 (9.8)	10 (19.2)	0.034
Der f 13	31 (19.4)	3 (4.5)	15 (36.6)	13 (25.0)	<0.001
Der f 14	35 (21.9)	5 (7.5)	17 (41.5)	13 (25.0)	<0.001
Der f 30	17 (10.6)	3 (4.5)	5 (12.2)	9 (17.3)	0.074
Der f 32	40 (25.0)	5 (7.5)	18 (43.9)	17 (32.7)	<0.001
Der f Alt a 10	29 (18.1)	2 (3.0)	10 (24.4)	17 (32.7)	<0.001

AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis

In addition, the number of allergens that patients were sensitized to differed among the groups. Patients suffering from AD were sensitized to a greater number of house dust mite component allergens that those with AA or AR only (Figure 6A). The majority (80.6%) of patients with AA/AR were sensitized to up to 2 allergens (Figure 6B), whereas 54% of AD patients showed reactivity to 3 to 5 allergens.

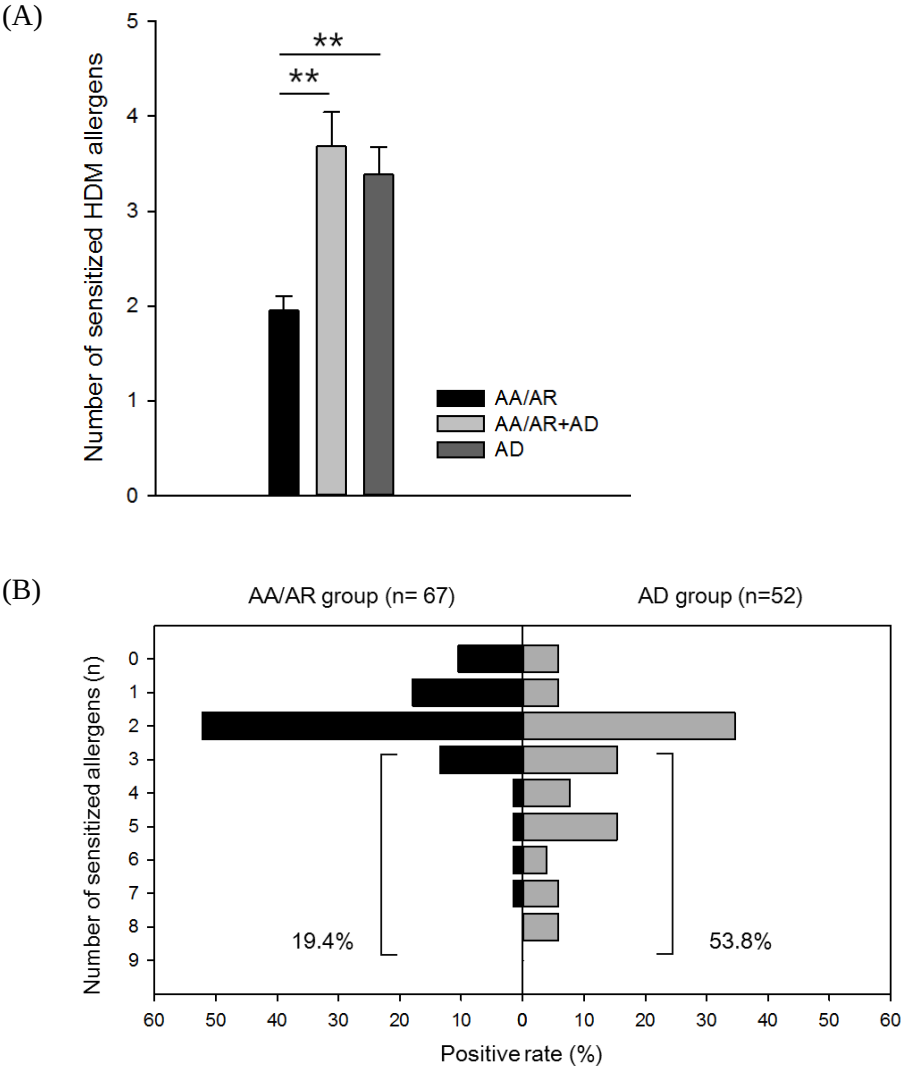


Figure 6. Number of sensitized component allergens in AA/AR and AD groups. (A) Number of sensitized component allergens in samples from each disease group. (B) Distribution of number of sensitized allergens compared between airway and dermatitis allergy groups. AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis. *P < .05, ** P < .01

Consistent with the sIgE titers measured by ImmunoCAP, the optical densities of the major allergens (Der f 1 or Der f 2) measured by ELISA were highest in the AD group (Figure 7), as were sIgE reactivities for Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10. Interestingly, patients sensitized to any of the minor allergens showed a tendency to be sensitized to multiple HDM component allergens, including Der f 11, Der f 13, and Der f Alt a 10 (see Figure 8).

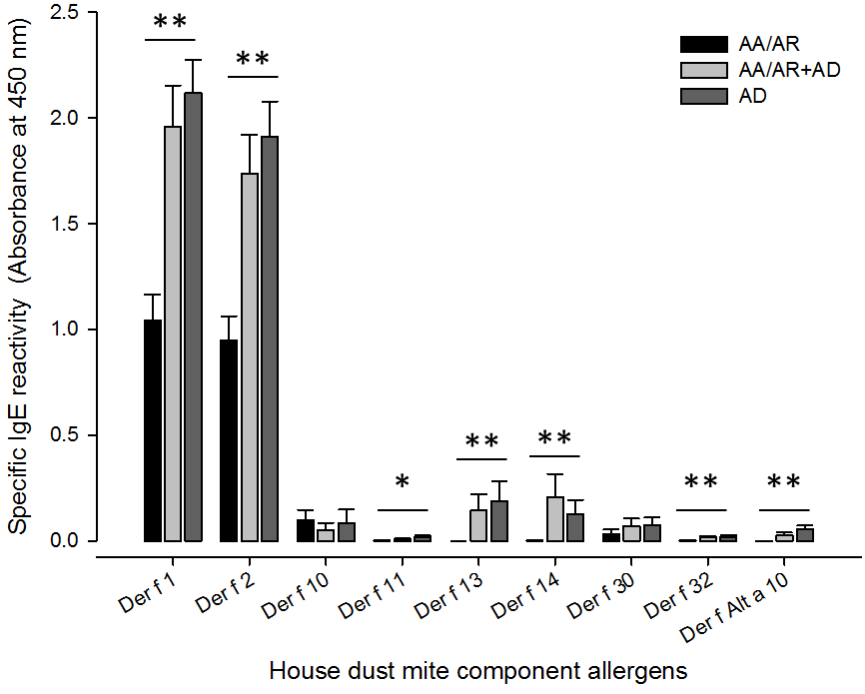


Figure 7. Specific IgE reactivities to component allergens in each disease group. AA, allergic asthma; AR, allergic rhinitis; AD, atopic dermatitis. *P < .05, ** P < .01

Figure 8. Percentage of multiple sensitizers per sensitized component allergens.

IV. DISCUSSION

In this study, the sensitization profiles of allergic patients to nine recombinant mite allergens were analyzed. Interestingly, patients who suffer from AD showed broader IgE recognition to multiple HDM component allergens and higher sIgE reactivities. On the basis of these results, we propose that Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10 can be used as immunologic markers for AD and can be considered for decisions involving the diagnosis and treatment of HDM allergies. Furthermore, patients' sensitization profiles for these allergens may be useful for monitoring and predicting clinical manifestations during the process of allergic march, which may affect diagnostic accuracy and immunotherapy.

Although the IgE binding frequencies for Der f 13, Der f 14, and Der f 32 have

been reported previously, we revealed that sensitization to these allergens, as well as to Der f Alt a 10, differs in a clinically relevant manner. Specifically, patients with AD showed higher rates of sensitization to these four allergen components. Moreover, Der f 10, Der f 11, Der f 13, Der f 14, Der f 30, Der f 32, and Der f Alt a 10 can be used as multi-sensitization markers, as patients with a sensitization to one also showed sensitization to at least one of the others. Of the other minor HDM allergens, Der p 10 has been the best studied with regard to clinical implications. Resch et al.[21] reported that Der p 10 is also a diagnostic marker for broader sensitization to other minor HDM component allergens. Overall, the sensitization rate to Der p 10 was 15.2% in that study, which is similar to that found in our study (14.4% overall). Another study found that the sensitization to Der p 10 and Der p 4 correlated with the severity of AD.[22] Pittner et al.[23] suggested a prediction model for HDM immunotherapy using a HDM component-based diagnosis, indicating that immunotherapy would be less effective in Der p 1-negative and Der p 10-positive patients. Der f 14 is known protease-sensitive allergen, and its breakdown products have higher allergenicity than the intact allergen.[20,24] Consistent with this, our data show that the sensitization to Der f 14 was higher in AD patients than in respiratory allergy patients.

The findings reported here are in line with a recent study showing that sensitization to Der p 11, a paramyosin protein localized in the muscles of HDM, was more strongly associated with AD than with respiratory allergies.[25] In this

previous study, Banerjee et al. proposed Der p 11, in addition to Der p 10, Der p 14, and Der p 18, as a surrogate marker for the discrimination of mite-associated AD patients from mite-allergic patients from four European countries and one African country. The results from the present study validate these findings in Asian patients and expand the list to include additional candidate allergens.

The higher prevalence for sensitization to minor allergens in patients with AD can be explained by the sensitization route. As a result of damage to the skin barrier, AD patients are more likely to sensitize to the body debris of dead HDM, which is the primary source of minor allergens. Moreover, HDM, as well as cockroaches and *Staphylococcus aureus*, are known to produce various proteases,[14] which can aggravate skin defects and more easily penetrate the skin barrier.[26,27] By contrast, HDM allergens excreted in fecal pellets (major group 1 and 2 allergen source) can be airborne and more easily enter the nostrils and airways to contribute to AR or asthma.[28]

The group 23 allergen was not detected in this study, though it is one of the serodominant allergens, along with group 1 and group 2 HDM allergens.[29] However, Der f 23 may not be a primary allergen in South Korea, as we found only one report describing group 23 mite allergens in Korea, in which Der f 23 was recognized by antibodies in sera from 42.8% of participants, whereas Der f 2 was recognized by 96.4%.[30]

Der f Alt a 10 is not listed by the WHO/IUIS Allergen Nomenclature

Sub-Committee as a *D. farinae*-derived allergen. To our knowledge, this is the first report describing Der f Alt a 10 as an allergen and its IgE reactivity. This allergen is homologous to fungal allergen, Alt a 10, which is an alcohol dehydrogenase derived from *Alternaria alternata*. Alt a 10 binds IgE in 2% of *Alternaria*-sensitized patients, but its clinical significance were not known.[31,32] In the present study, more patients with AD were sensitized to Der f Alt a 10 (32.7%) than patients in the AA/AR group (3.0%).

It is important to note that the compositions of *D. farinae* allergen extracts may vary by pharmaceutical company or by batch, as they are prepared directly from HDM. The policies regarding the production of raw materials (purified HDM body or feces or both) and the standardization methods differ in the United States and in Europe.[33,34] The ratio of group 1/group 2 allergens in commercial *D. farinae* extracts can range from 0.9 to 20.5.[28] Unfortunately, the compositions and concentrations of minor allergens in HDM extracts are largely unknown. IgE reactivities to HDM minor allergens (Der f 4, Der f 5, Der f 6, Der f 13, Der f 15, Der f 21, and Der f 23) in 1,302 serum samples from America, Canada, Europe, and Japan range from 20 to 47%.[2] Thus, further studies on the minor mite allergens are crucial for clarifying the variability in extract compositions and verifying the sensitivities for these components in various allergic diseases.

There are several limitations of this study. First, IgE detection was analyzed by using Western blots and ELISAs. Inhibition tests are needed to confirm IgE

binding affinity and cross-reactivity. Unfortunately, such experiments were not conducted due to the limited serum samples available in this study. Second, patients from a single hospital in the Republic of Korea were analyzed, and the results should be confirmed in further multicenter and multinational studies. Third, despite the cross-reactivity between *D. farinae* and *D. pteronyssinus*, we produced recombinant component allergens from *D. farinae* only, as this is the dominant species in Seoul. Fourth, we did not examine disease severity or causal relationships between reactivities and allergic march, which should be assessed in prospective cohort studies.

V. CONCLUSION

We identified sensitization profiles to HDM component allergens in patients with respiratory and cutaneous allergic diseases. We found that patients with AD were sensitized to a broader range of minor HDM component allergens (Der f 11, Der f 13, Der f 14, Der f 32, and Der f Alt a 10), whereas patients with respiratory allergies were sensitized to the major components. The findings revealed in this study may be applicable to the selection of appropriate therapeutic agents for HDM immunotherapy and to predict their therapeutic effectiveness.

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ABSTRACT (IN KOREAN)

집먼지진드기 알레르기 환자에서 질환 별 집먼지진드기 성분항원 감작패턴 비교

<지도교수 박 중 원 >

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박 경 희

연구배경: 집먼지진드기는 천식, 비염, 아토피피부염 등 다양한 알레르기 질환의 원인이다. 집먼지진드기는 다양한 성분 항원으로 구성되어있으나, 그 항원의 알레르기성, 임상적 함의는 널리 알려져 있지 않다. 본 연구를 통해 알레르기 면역반응과 관련된 집먼지진드기의 성분 항원을 밝히고, 알레르기 환자에서 임상 표현형에 따른 성분 항원의 감작 패턴을 파악하고, 비교하고자 하였다.

연구방법: 160명의 집먼지진드기 알레르기 환자에서 진단명에 따른 집먼지진드기 성분 항원의 감작 패턴 분석을 분석하였다. IgE 반응성은 일차원 및 이차원 전기영동과 면역블로팅으로 분석하였다. 질환에 따라 면역블로팅에서 차이가 나는 단백질의 아미노산 서열을 액체 크로마토 그래피 – 결합 전기 분무

이온화 - 탠덤 질량 분석으로 확인하였다. 이를 바탕으로 Der f 1, Der f 2, Der f 10, Der f 11, Der f 13, Der f 14, Der f 30, Der f 32 및 Der f Alt a 10과 같은 9 개의 재조합 진드기 알레르겐이 합성하였고, IgE 각각에 대한 혈청 반응성은 ELISA 기법으로 측정하였다.

연구결과: 전체 환자 중 88.1%와 78.1% 의 혈청 시료가 Der f 1과 Der f 2 에 각각 IgE 반응을 하였다. 호흡기 알레르기 환자는 주로 Major 알레르겐에 감작되어있었지만, 아토피피부염 환자는 Major 알레르겐 성분 및 Minor 알레르겐 성분에 대해 다제 감작을 나타냈다 (Der f 11, Der f 13, Der f 14, Der f 32 및 Der f Alt a 10).

결론: 집먼지진드기에 의한 호흡기 알레르기 환자 (비염, 천식)은 주로 Major 성분 항원에 감작되어 있으며, 피부 알레르기 환자 (아토피피부염) 는 minor 성분 항원 (Der f 11, Der f 13, Der f 14, Der f 32, Der f Alt a 10) 에 감작된 패턴을 보였다. 따라서 본 연구 결과는 성분항원을 이용한 진단법 및 예후 예측, 알레르겐 특이 면역 치료선정의 근거 자료로 활용할 수 있다.

핵심되는 말 : 집먼지진드기, 알레르겐, 성분 항원, 재조합 항원, 알레르기비염, 천식, 아토피피부염, 특이 IgE