Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2016;170:132–137
DOI: 10.1159/000447694

Received: February 29, 2016
Accepted after revision: June 15, 2016
Published online: August 10, 2016

# Real-Life Study for the Diagnosis of House Dust Mite Allergy – The Value of Recombinant Allergen-Based IgE Serology

Sven Becker[a, b] Thomas Schlederer[c] Matthias F. Kramer[b] Mareike Haack[b] Susanne Vrtala[d] Yvonne Resch[d] Christian Lupinek[d] Rudolf Valenta[d] Moritz Gröger[b]

[a]Department of Otorhinolaryngology and Head and Neck Surgery, University Medical Center, Johannes Gutenberg University, Mainz, and [b]Department of Otorhinolaryngology and Head and Neck Surgery, University of Munich, Munich, Germany; [c]Thermo Fisher Scientific, and [d]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Vienna, Austria

**Key Words**

Allergen components · Allergic rhinitis · Der p 1 · Der p 2 · Der p 23 · *Dermatophagoides pteronyssinus* · House dust mites · Major allergens

**Abstract**

***Background:*** *Dermatophagoides pteronyssinus* is one of the most important perennial allergen sources worldwide. Molecular diagnostics using the commercially available major allergens (Der p 1 and Der p 2) in combination with Der p 10 do not detect house dust mite (HDM) sensitization in a number of cases when used alone. The objective was to evaluate the IgE reactivity profiles of these patients using an experimental immunoassay biochip. ***Methods:*** Sera of HDM-allergic patients (positive skin prick test, CAP class ≥1 for allergen extract, and positive intranasal provocation) were tested for IgE antibodies against Der p 1, Der p 2, and Der p 10 by ImmunoCAP fluorescence enzyme immunoassay. Negatively tested sera were examined by an experimental chip containing 13 microarrayed HDM allergens. ***Results:*** Of 97 patients tested, 16 showed negative results to Der p 1, Der p 2, and Der p 10. MeDALL chip evaluation revealed 5 patients monosensitized to Der p 23, and 11 patients were negative for all HDM MeDALL chip components. Seven sera were available for further testing, and 3 of them showed IgE reactivity to dot-blotted nDer p 1, and 2 reacted with high-molecular weight components (>100 kDa) in nitrocellulose-blotted HDM extract when tested with $^{125}$I-labeled anti-IgE in a RAST-based assay. The HDM extract-specific IgE levels of the 11 patients were <3.9 kU/l. ***Conclusions:*** Recombinant allergen-based IgE serology is of great value when conventional IgE diagnostics fails. Der p 23 is an important HDM allergen, especially when major allergens are negative. Therefore, it would be desirable to have Der p 23 commercially available. Further research concerning the prevalence and clinical significance of different HDM allergens is needed.



## Introduction

House dust mites (HDM), especially *Dermatophagoides pteronyssinus*, are the most important perennial allergen sources in central Europe and cause allergic rhinitis as well as allergic asthma [1]. So far, more than 20 differ-

KARGER

E-Mail karger@karger.com
www.karger.com/iaa


1018–2438/16/1702–0132$39.50/0

Correspondence to: Dr. med. Sven Becker
Department of Otorhinolaryngology and Head and Neck Surgery
University Medical Center, Johannes Gutenberg University
Langenbeckstrasse 1, DE–55101 Mainz (Germany)
E-Mail sven.becker@unimedizin-mainz.de

ent allergens of *D. pteronyssinus* have been described, with a strong variation in prevalence rates for the major allergens Der p 1 and Der p 2 in different countries [2–5]. The third currently commercially available allergen component Der p 10 also showed varying prevalence rates being responsible for a part of the cross-reactions to arthropods and mollusks [5, 6].

Molecular allergy diagnostics has become an important tool in daily allergological routine in allergy centers but also in smaller facilities within the last decade. The knowledge about major and minor allergen components and their prevalence opened new possibilities in allergy diagnostics and therapy. This powerful tool can often explain the sometimes frustrating outcomes of immunotherapy in the past and helps to choose the right treatment option (symptomatic vs. immunotherapy) for the individual patient. In comparison to immunotherapy against grass or birch pollen, immunotherapy against HDM shows considerably lower success rates [7, 8]. One reason for this reduced therapeutic success might be the fact that most commercially available therapeutic agents for immunotherapy of HDM allergy are standardized to the major allergens Der p 1, Der p 2, and Der f 1 [9, 10], thereby not matching for patients sensitized to other components. Up to now, more than 20 allergen components of *D. pteronyssinus* with a varying prevalence and importance for therapy have been described. Weghofer et al. [11] showed a high allergenic activity of Der p 23. This new allergen component is localized in the peritrophic matrix lining the midgut of *D. pteronyssinus* and also the surface of fecal pellets. Due to its association with mite feces, it becomes airborne and respirable, which might be a reason for its impact on the development of HDM allergy.

The aim of the current study was to investigate retrospectively the value of recombinant allergen-based IgE serology using an experimental allergen chip (MeDALL chip) in HDM-allergic patients tested negative for the commercially available HDM components Der p 1, Der p 2, and Der p 10 [12]. The results may identify components which can improve diagnostics and therapy of HDM allergy in the future.

## Patients and Methods

Patient data were selected from the allergy database of the Department of Otorhinolaryngology, and Head and Neck Surgery of the Ludwig Maximilians University in Munich where all relevant diagnostic results of patients are stored. The study was approved by the local ethics committee and the local data protection commissioner. All patients gave informed consent. The database was scanned for consecutive patients with a proven perennial allergy to HDM who presented at our institution between 2001 and 2010. The diagnosis of HDM allergy was based on the following selection criteria: (1) A positive skin prick test for *D. pteronyssinus*. The skin prick test (ALK-Abelló, Wedel, Germany) was read after 20 min. (2) CAP class ≥1 [≥0.35 $kU_A$/l; ImmunoCAP fluorescence enzyme immunoassay (FEIA); Thermo Fisher Scientific, Freiburg, Germany] for the HDM *D. pteronyssinus*. (3) Positive intranasal provocation with a standard provocation solution (*D. pteronyssinus*; ALK-Abelló) according to guideline specifications [13] (decrease in rhinomanometry >40% at 150 Pa on the allergen-challenged side or symptom score >3, or decrease in rhinomanometry >20% at 150 Pa on the allergen-challenged side in combination with a symptom score >2). The provocation solution contained 200,000 SQ units/ml or 19.6 µg/ml Der p 1 [pers. notification Prof. Dr. E. Wüstenberg, ALK-Abelló]. (4) Availability of the patient's serum at our allergy serum bank.

Nasal symptoms were semiquantitatively assessed using four categories on the day of the first visit: (1) obstruction, (2) rhinorrhea, (3) sneezing, and (4) itching. Each symptom could be answered between 0 and 3: 0 = no impairment, 1 = mild impairment, 2 = moderate impairment, 3 = severe impairment.

*Fluorescence Enzyme Immunoassay*

Sera of the patients which fulfilled the above-mentioned criteria were analyzed for specific IgE antibodies to nDer p 1, rDer p 2, and rDer p 10 using the FEIA method with a commercial test kit (ImmunoCAP-FEIA, Thermo Fisher Scientific). Results were stated in CAP classes as well as in concentrations (kU/l).

*MeDALL Allergen Chip Analysis*

Sera of patients with a negative result to nDer p 1, rDer p 2, and rDer p 10 were examined for specific IgE antibodies to cross-reactive carbohydrate determinants (CCDs) and then transferred to ThermoFisher-Phadia multiplexing, Vienna, Austria, for MeDALL chip analysis.

The MeDALL allergen chip is based on the latest allergen microarray technology for diagnosis and monitoring of IgE and IgG reactivity profiles of allergic patients which was developed within the FP 7-funded European Union project MeDALL in collaboration with the Division of Immunopathology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, and ThermoFisher-Phadia multiplexing. Beside a broad variety of different allergen groups, the chip contains a panel of 13 components from *D. pteronyssinus* (Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, and Der p 23) and clone 16-encoded allergen. Sera from patients fulfilling the above-mentioned criteria were examined by the chip as described [12].

*Statistical Analysis*

Statistical analysis was performed with SigmaStat and SigmaPlot 2000 for Windows version 6.00 (Jandel Corp., San Rafael, Calif., USA). To compare nasal symptoms, normally distributed data were tested by t test and given as means ± SD. To compare non-normally distributed data, the Mann-Whitney rank sum test was used, and results are given as medians and ranges. To examine the correlation of two variables, the Pearson product correlation coefficient was used.

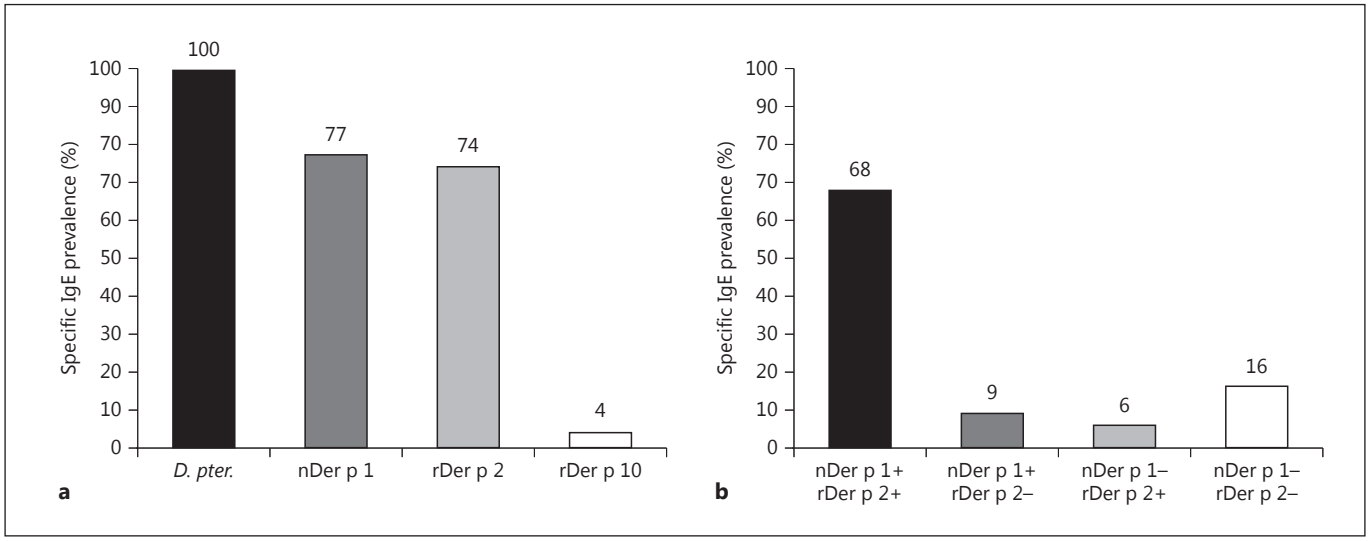


**Fig. 1. a** Prevalence of IgE reactivity to *D. pteronyssinus (D. pter.)*, nDer p 1, rDer p 2, and rDer p 10 in proven HDM-allergic patients in southern Bavaria (n = 97). **b** Prevalence rates for different combinations of the two major allergens Der p 1 and Der p 2. Sixteen patients negative for the two allergen components had also negative results for Der p 10. Adapted from [4], with kind permission of Springer publishing house.

## Results

The database query with the above-mentioned inclusion criteria resulted in 97 patients with a proven allergy to HDM. Prevalence rates for *D. pteronyssinus*, nDer p 1, rDer p 2, and rDer p 10 were 100, 77, 74 and 4%, respectively. IgE concentrations were 15.69 ± 21.33, 10.40 ± 15.85, 17.63 ± 24.76 and 0.89 ± 0.80 kU/l (means ± SD) respectively. IgE levels to HDM extract *D. pteronyssinus* correlated well with the sum of IgE levels of Der p 1, Der p 2 and Der p 10. Pearson's correlation coefficient r = 0.851, $p < 0.05$, n = 81. Sixteen patients showed negative results to all three components (table 1; fig. 1). Within this group, gender distribution showed 9 female (56%) and 7 male (44%) patients with a mean age of 34.1 (14.7 SD) years and a range between 11 and 60 years. Nine patients (56%) showed a single sensitization to HDM, 7 patients (43%) showed additional sensitization to further aeroallergens.

**Table 1.** Patient demographics and study results

| | |
|---|---|
| All patients, n (%) | 97 (100) |
| Mean age, years (range) | 30 (8–66) |
| Gender, n (%) | |
| Male | 56 (58) |
| Female | 41 (42) |
| Monosensitized to HDM, n (%) | 30 (31) |
| Polysensitized, n (%) | 61 (63) |
| No data available, n (%) | 6 (6) |
| Nasal symptoms, means ± SD | |
| Obstruction | 1.95±1.07 |
| Rhinorrhea | 1.41±1.00 |
| Sneezing | 1.32±0.96 |
| Itching | 1.12±0.93 |

Nasal symptoms were semiquantitatively assessed on the day of the first visit on a scale from 0 (no impairment) to 3 (severe impairment).

MeDALL chip evaluation of these 16 sera tested positive to *D. pteronyssinus* but negative to Der p 1, Der p 2, and Der p 10 resulted in 5 patients (31%) sensitized to Der p 23. All other HDM allergens on the chip (Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, and Der p 21, and clone 16) were negative. Negative test results were also seen for the two major allergen components of *D. farinae* Der f 1 and Der f 2 in these 16 patients. Examination of specific IgE antibodies to CCDs showed negative results in 12 patients, 2 patients showed positive results (1 CAP class 2 and 1 CAP class 1). Both patients were polysensitized with high concentrations of specific IgE to birch, grass, and other plants. In 2 patients, there was no serum left for this examination. Detailed information about the 16 patients who underwent MeDALL chip evaluation can be found in table 2. In

**Table 2.** Detailed evaluation of the 16 HDM-allergic patients who were negative for Der p 1, 2, and 10 by conventional IgE serology

| Patient | Age, years | Obstruction | Rhinorrhea | Sneezing | Itching | SPT *D. pter.* | SPT *D. far.* | SPT histamine | sIgE *D. pter.*, kUA/l | sIgE *D. far.*, kUA/l | CCD, kUA/l | NPT rhinomanometry (decrease) | NPT symptom score | NPT evaluation | Sensibilization | MeDALL Der p 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C.L.** | **11** | **1** | **0** | **2** | **0** | **2** | **0** | **2** | **1.52** | **0.69** | **0.00** | + | – | + | **M** | + |
| **A.S.K.** | **21** | **3** | **2** | **0** | **0** | **2** | **1** | **3** | **1.44** | **0.35** | **0.00** | – | + | + | **P** | + |
| O.C. | 53 | 2 | 2 | 3 | 3 | 1 | 1 | 3 | 0.38 | 0.35 | 0.00 | + | n.a. | + | M | – |
| **S.H.** | **37** | **3** | **2** | **1** | **3** | **2** | **1** | **3** | **1.15** | **0.35** | **0.00** | + | + | + | **P** | + |
| **D.S.** | **18** | **1** | **2** | **1** | **2** | **3** | **3** | **3** | **0.37** | **0.39** | **0.35** | – | + | + | **M** | + |
| G.R. | 60 | 2 | 1 | 3 | 2 | 1 | 1 | 3 | 0.72 | 0.56 | 0.00 | – | + | + | P | – |
| **M.B.** | **27** | **n.a.** | **n.a.** | **n.a** | **n.a.** | **3** | **0** | **3** | **2.99** | **0.59** | **1.60** | **n.a.** | **n.a.** | + | **P** | + |
| K.L. | 56 | 3 | 1 | 1 | 2 | 2 | 0 | 3 | 0.67 | 0.50 | 0.00 | n.a. | n.a. | + | M | – |
| H.H. | 27 | n.a. | n.a. | n.a. | n.a. | 3 | 1 | 3 | 3.84 | 3.69 | 0.00 | + | – | + | M | – |
| J.K.K. | 23 | n.a. | n.a. | n.a. | n.a. | 2 | 2 | 3 | 0.45 | 0.39 | 0.00 | – | + | + | M | – |
| E.F. | 27 | 1 | 0 | 0 | 0 | 2 | 2 | 3 | 1.40 | 1.32 | 0.00 | – | + | + | P | – |
| A.K. | 29 | n.a. | n.a. | n.a. | n.a. | 1 | 1 | 3 | 0.46 | 0.40 | 0.00 | – | + | + | M | – |
| S.R.S. | 38 | 2 | 2 | 1 | 1 | 2 | 2 | 3 | 0.85 | 0.66 | 0.00 | + | + | + | P | – |
| J.T. | 56 | 2 | 2 | 1 | 1 | 2 | 2 | 3 | 0.74 | 0.68 | 0.00 | – | + | + | M | – |
| H.W. | 36 | 3 | 2 | 2 | 1 | 1 | 1 | 3 | 0.75 | 0.86 | 0.00 | – | + | + | M | – |
| S.M. | 29 | 0 | 1 | 1 | 0 | 1 | 1 | 3 | 1.41 | 1.61 | 0.00 | – | + | + | P | – |
| Mean | 34.25 | 1.92 | 1.42 | 1.33 | 1.25 | 1.88 | 1.19 | 2.94 | 1.20 | 0.84 | | | | | | |
| SD | 14.84 | 1.00 | 0.79 | 0.98 | 1.14 | 0.72 | 0.83 | 0.25 | 0.96 | 0.84 | | | | | | |
| Max. | 60.00 | 3.00 | 2.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.84 | 3.69 | | | | | | |
| Min. | 11.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 2.00 | 0.37 | 0.35 | | | | | | |

Patients who had a positive result on the MeDALL chip (Der p 23) are shown in bold. Nasal symptoms (obstruction, rhinorrhea, sneezing, and itching) were semiquantitatively assessed on the day of the first visit: 0 = no impairment, 1 = mild impairment, 2 = moderate impairment, 3 = severe impairment. Skin prick test (SPT): 0 = wheal <3 mm, 1 = wheal 3–4 mm, 2 = wheal 4–5 mm, 3 = wheal 5–6 mm, 4 = wheal >6 mm. Nasal provocation test (NPT): decrease in rhinomanometry >40% at 150 Pa on the allergen-challenged side or symptom score >3, or decrease in rhinomanometry >20% at 150 Pa on the allergen-challenged side and symptom score >2. *D. pter.* = *D. pteronyssinus*; *D. far.* = *D. farinae*; M = monosensitized; P = polysensitized; n.a. = not available; Max. = maximum; Min. = minimum.

the 5 Der p 23-positive patients, specific IgE levels to HDM extract (measured in kU/l) and MeDALL chip results to Der p 23 (measured in ISAC standardized units) tend to increase together with Pearson's correlation coefficient r = 0.808. Unfortunately, correlation was not significant (p = 0.084) due to the low number of patients (n = 5). The symptoms of these patients did not differ from those of the entire cohort of 97 HDM-allergic patients [obstruction median 2.00 (range 0.00–3.00) vs. 2.00 (0.00–3.00), p = 0.69; rhinorrhea 2.00 (0.00–2.00) vs. 1.00 (0.00–3.00), p = 0.96; sneezing 1.00 (0.00–3.00) vs. 1.00 (0.00–3.00), p = 0.99, and itching 1.00 (0.00–3.00) vs. 1.00 (0.00–3.00), p = 0.93] nor did Der p 23-positive in comparison to Der p 23-negative patients [obstruction 2.00 ± 1.16 (mean ± SD) vs. 1.88 ± 0.99, t(10) = 0.20, p = 0.85; rhinorrhea 2.00 (0.00–2.00) vs. 1.50 (0.00–2.00), p = 0.68; sneezing 1.00 ± 0.82 vs. 1.50 ± 1.07, t(10) = 0.82, p = 0.43, and itching: 1.25 ± 1.50 vs. 1.25 1.04, t(10) = 0.00, p = 1.00].

For the 11 sera which were tested negative for all allergen components, a correlation was calculated between the concentrations of specific IgE antibodies to the extracts of *D. pteronyssinus* and *farinae*, resulting in a correlation coefficient of 0.992, p < 0.0001.

From 7 patients, sera were available for further testing of which 3 sera showed IgE reactivity to dot-blotted nDer p 1, and 2 others reacted with high-molecular weight components (>100 kDa) in nitrocellulose-blotted HDM extract when tested with $^{125}$I-labeled anti-IgE in a RAST-based assay (data not shown). The HDM extract-specific IgE levels of the 11 patients were <3.9 kUA/l (table 2).

## Discussion

By means of the actually available HDM allergen components (Der p 1, Der p 2, and Der p 10), it was not possible to diagnose HDM allergy in a number of patients when used alone. Research groups from central Europe, Africa, Brazil, and Australia have published different IgE reactivity patterns in HDM-allergic patients, demonstrating that there is no single major allergen component that can be used for diagnostic purposes alone worldwide [3–5, 14–17]. Therefore, the aim of this study was to analyze

the reactivity profile of proven HDM-allergic patients which were tested negative for the commercially available allergens Der p 1, Der p 2, and Der p 10 with an experimental allergen chip containing additional 10 HDM allergen components. Special interest was focused on Der p 23 as Weghofer et al. [11] showed in 347 patients a comparable prevalence of Der p 23 (74%) in comparison to Der p 1 and Der p 2 indicating the importance of this allergen component as a new major HDM allergen. This fact is supported by latest research from Thailand where 54% of 222 HDM-allergic patients displayed Der p 23-specific IgE responses [18]. In the current study, 5 of 16 patients showed positive results for Der p 23 whereas all other allergen components including Der p 4, which showed a high prevalence in an aboriginal community in Australia [19], were tested negative. Symptoms of Der p 23-positive patients did not differ from symptoms of patients who tested negative for this component. Der p 23 therefore may help to clarify HDM allergy in nearly one third of the cases when until now commercially available HDM component testing leads to negative results in the catchment area of our hospital in southern Germany.

Cross-reacting group 1 and group 2 allergens from *D. pteronyssinus* and *farinae* are well known [20]. In all the 16 patients, Der f 1 and Der f 2 as well as Der p 1 and Der p 2 showed negative results in immunoassay biochip testing in spite of positive results for allergen extracts of *D. pteronyssinus* and *farinae* by FEIA. In patients tested negative for all HDM components, concentrations of IgE antibodies against both allergen extracts correlated well, indicating a cross-reacting allergen component which is not covered by the MeDALL chip so far. Chan et al. [21] were able to identify a new allergen component of *D. farinae* (Der f 24), an ubiquinol-cytochrome c reductase binding protein originating from *Enterobacter* species inhabiting the mite gut. The authors highlight the fact that the internal HDM body is host to more than 100 bacterial species, with a predominance of *Enterobacter* species, and that allergen components from their microbiome can be of importance for HDM allergenicity and immunotherapy mechanisms. Although not proven yet, the results of Chan et al. [21] might explain our findings for group 1 and 2 allergens from *D. pteronyssinus* and *farinae* via reactions to bacterial allergen components which can be found in both allergen extracts.

CCDs from plants and invertebrates are able to induce IgE production with cross-reacting properties [22–24]. CCD sensitization is normally considered clinically irrelevant due to a poor activity in vivo but can serve as a disturbing factor in specific IgE assays especially in patients allergic to plants and in patients with a *Hymenoptera* venom allergy [25, 26]. Only 2 of 15 patients in the current study showed positive results for CCDs indicating that the good correlation between the allergen extracts of *D. pteronyssinus* and *farinae* is not caused by these cross-reacting antibodies.

Subcutaneously or sublingually applied allergen-specific immunotherapy represents the only disease-modifying and allergen-specific approach with long-lasting effects. In contrast, symptomatic therapy is only able to decrease symptoms like nasal obstruction, rhinorrhea and itching but cannot influence the allergic immune response itself. From this perspective, immunotherapy is superior to symptomatic therapy. In our study, 11 patients (11%) showed negative results for the previous HDM major allergens Der p 1 and Der p 2 but additionally showed negative results for further 10 HDM allergens when using the experimental MeDALL chip. Most companies in the market of HDM immunotherapy standardize their formulations to Der p 1, Der p 2, or Der f 1 [9, 10]. Analysis of HDM extracts from different manufacturers revealed varying allergen compositions and contents when examined for several *D. pteronyssinus* allergen components (Der p 1, 2, 5, 7, 10, and 21) by Casset et al. [27]. Der p 1 and Der p 2 could be detected in all extracts, but high variations in concentrations were seen, whereas other components, e.g. Der p 21, were completely absent in many formulations. Consequently, patients with a proven allergy due to one of these two major allergen components should benefit from therapy. But what should be done with patients not sensitized to theses major components, showing sensitization to Der p 23 or the HDM extract only? Are these patients suitable for immunotherapy with the actually available therapeutic solutions? These questions should be addressed in the future to improve clinical results of immunotherapy and to reduce treatment failure. Eleven of the 97 patients (11%) in our study showed a reactivity profile with none of 13 HDM allergen components in commercially available test platforms. As a consequence of these findings, molecular diagnostics for major allergen components should be mandatory before immunotherapy in HDM-allergic patients. For diagnostic purposes, measurement of specific antibodies to HDM extracts is a safe and routine procedure. To optimize therapy outcome and to improve reporting and comparability of clinical trials, it would be desirable to have more information about the content of major and minor allergen components in therapeutic solutions used in daily routine as well as in clinical trials.

Der p 23 is a new important HDM allergen which can help to clarify perennial allergic symptoms when the known major HDM allergen components Der p 1 and Der p 2 as well as Der p 10 were negative. Further research concerning prevalence and clinical relevance of Der p 23 is needed to improve diagnostics and therapy of house dust mite allergy in the future. It is desirable to have Der p 23 commercially available for research purposes as well as in clinical routine.


## Acknowledgment

This study was supported in part by grants F4602 and F4605 of the Austrian Science Fund (FWF) and by the Christian Doppler Association, Vienna, Austria.


## References


1 Bousquet J, Lockey R, Malling HJ, Alvarez-Cuesta E, Canonica GW, Chapman MD, et al: Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998;81:401–405.

2 Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, et al: Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004;34:597–603.

3 Westritschnig K, Sibanda E, Thomas W, Auer H, Aspock H, Pittner G, et al: Analysis of the sensitization profile towards allergens in central Africa. Clin Exp Allergy 2003;33:22–27.

4 Becker S, Kramer MF, Havel M, Welz C, Markmann S, Groger M: IgE reactivity profiles among house dust mite allergic patients in Bavaria. Eur Arch Otorhinolaryngol 2013; 270:3177–3182.

5 Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al: Variability of IgE reactivity profiles among European mite allergic patients. Eur J Clin Invest 2008; 38:959–965.

6 Becker S, Groger M, Canis M, Pfrogner E, Kramer MF: Tropomyosin sensitization in house dust mite allergic patients. Eur Arch Otorhinolaryngol 2012;269:1291–1296.

7 Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S: Allergen injection immunotherapy for seasonal allergic rhinitis. Cochrane Database Syst Rev 2007; 1:CD001936.

8 Nelson HS: Update on house dust mite immunotherapy: are more studies needed? Curr Opin Allergy Clin Immunol 2014;14:542–548.

9 Calderon MA, Casale TB, Nelson HS, Demoly P: An evidence-based analysis of house dust mite allergen immunotherapy: a call for more rigorous clinical studies. J Allergy Clin Immunol 2013;132:1322–1336.

10 Meyer CH, Bond JF, Chen MS, Kasaian MT: Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. Clin Exp Allergy 1994;24: 1041–1048.

11 Weghofer M, Grote M, Resch Y, Casset A, Kneidinger M, Kopec J, et al: Identification of Der p 23, a peritrophin-like protein, as a new major *Dermatophagoides pteronyssinus* allergen associated with the peritrophic matrix of mite fecal pellets. J Immunol 2013;190:3059–3067.

12 Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al: Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. Methods 2014;66:106–119.

13 Riechelmann H, Bachert C, Goldschmidt O, Hauswald B, Klimek L, Schlenter WW, et al: Application of the nasal provocation test on diseases of the upper airways. Position paper of the German Society for Allergology and Clinical Immunology (ENT Section) in cooperation with the Working Team for Clinical Immunology (in German). Laryngorhinootologie 2003;82:183–188.

14 Bronnert M, Mancini J, Birnbaum J, Agabriel C, Liabeuf V, Porri F, et al: Component-resolved diagnosis with commercially available *D. pteronyssinus* der p 1, der p 2, and der p 10: relevant markers for house dust mite allergy. Clin Exp Allergy 2012;42:1406–1415.

15 Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM: Characterization and immunobiology of house dust mite allergens. Int Arch Allergy Immunol 2002;129:1–18.

16 Thomas WR, Smith WA, Hales BJ: The allergenic specificities of the house dust mite. Chang Gung Med J 2004;27:563–569.

17 Trombone AP, Tobias KR, Ferriani VP, Schuurman J, Aalberse RC, Smith AM, et al: Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. Clin Exp Allergy 2002;32:1323–1328.

18 Soh WT, Le Mignon M, Suratannon N, Satitsuksanoa P, Chatchatee P, Wongpiyaboron J, et al: The house dust mite major allergen Der p 23 displays *O*-glycan-independent IgE reactivities but no chitin-binding activity. Int Arch Allergy Immunol 2016;168:150–160.

19 Hales BJ, Laing IA, Pearce LJ, Hazell LA, Mills KL, Chua KY et al: Distinctive immunoglobulin E anti-house dust allergen-binding specificities in a tropical Australian Aboriginal community. Clin Exp Allergy 2007;37:1357–1363.

20 Yasueda H, Mita H, Yui Y, Shida T: Comparative analysis of physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae*. Int Arch Allergy Appl Immunol 1989;88:402–407.

21 Chan TF, Ji KM, Yim AK, Liu XY, Zhou JW, Li RQ, et al: The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. J Allergy Clin Immunol 2015;135: 539–548.

22 Malandain H: IgE-reactive carbohydrate epitopes – classification, cross-reactivity, and clinical impact (2nd part). Eur Ann Allergy Clin Immunol 2005;37:247–256.

23 van Ree R: Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002;129:189–197.

24 Altmann F: The role of protein glycosylation in allergy. Int Arch Allergy Immunol 2007; 142:99–115.

25 Vidal C, Sanmartin C, Armisen M, Rodriguez V, Linneberg A, Gonzalez-Quintela A: Minor interference of cross-reactive carbohydrates with the diagnosis of respiratory allergy in standard clinical conditions. Int Arch Allergy Immunol 2012;157:176–185.

26 Malandain H, Giroux F, Cano Y: The influence of carbohydrate structures present in common allergen sources on specific IgE results. Eur Ann Allergy Clin Immunol 2007;39: 216–220.

27 Casset A, Mari A, Purohit A, Resch Y, Weghofer M, Ferrara R, et al: Varying allergen composition and content affects the in vivo allergenic activity of commercial *Dermatophagoides pteronyssinus* extracts. Int Arch Allergy Immunol 2012;159:253–262.