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Sensitisation to molecular components of mites in atopic dermatitis patients

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ABSTRACT

Background and aim: To analyse the sensitisation profile to molecular components of mites allergens with the use of ALEX2 Allergy Explorer test in Atopic dermatitis (AD) patients. **Method:** The complete dermatological and allergological examination including the examination of the sensitisation to molecular components with ALEX2 Allergy Explorer testing was performed. For the statistical analysis, we used Fisher's Exact. **Results and conclusion:** The sensitisation to mites increases with AD severity. The central role in atopic march may play the molecular component Der f 2 and Der p 23. The results of our study confirm the important role of Der p 5 and Der p 7 in subgroup of patients suffering from bronchial asthma. The specific immunotherapy should focus on the components of NPC2 family and Der p 23 in AD patients. Our results may be helpful in planning hyposensitisation, treatment and immunotherapy.

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KEYWORDS

Atopic dermatitis; allergy to mites; sensitisation to molecular components; Der p 23

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder, characterised by intensely itchy skin. The prevalence of AD in industrialised countries has tripled during the past 30 years, affecting 15–30% of children and up to 10% of adults (Czarnowicki et al., 2017). Several factors seem to contribute to the development of AD including an impaired barrier function of atopic skin with higher skin pH and higher transepidermal water loss than healthy skin and an altered skin immune system with the altered production of antimicrobial peptides and cytokines (De Benedetto et al., 2012; Hönzke et al., 2016; Paller et al., 2019). AD is characterised by a biphasic inflammation, evolving from an initial, acute, Th2 and Th22-dominated phase to a chronic phase characterised by the concomitant presence of T helper Th1, Th2 cells and Th17 cells (Paller et al., 2019). A key player of innate immunity is the epidermal barrier and the loss-of-function *FLG* gene variants that constitute a major predisposing factor for AD (De Benedetto et al.,

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2012). A recent study indicates that *FLG*-deficiency renders skin equivalents more sensitive to the detrimental effects of IL-4 and IL-13 compared to skin equivalents with normal *FLG* expression, and therefore, defects in the epidermal barrier, skin permeability and cutaneous innate immune response are not primarily linked to *FLG* gene deficiency but are rather secondarily induced by Th2 inflammation (Hönzke et al., 2016).

Mites and ticks are included in the subclass Acari, which forms one of the most abundant and diverse biological groups within the arachnids (Arthropods). Mites found worldwide on human premises can generally be grouped into house dust mites (HDM) and storage mites (Sánchez-Borges et al., 2017). HDM are an unsurpassed cause of atopic sensitisation and allergic illness throughout the world. The main species from HDMs are *D. pteronyssinus*, *D. farinae*, *Euroglyphus maynei*. Storage mites (include *Lepidoglyphus destructor*, *Blomia tropicalis*, *Glycyphagus domesticus*, *Tyrophagus putrescentiae*, *Acarus siro*, *Aleuroglyphus ovatus*, *Suidasia medanensis* and *Thyreophagus entomophagus*) are global pests of stored food products of increasing medical and economical impact (Sánchez-Borges et al., 2017). The relationship between dust mites and AD was first described almost 30 years ago when a correlation was observed between the number of dust mites in the home and the presence and severity of AD in patients (Beck & Korsgaard, 1989; Sánchez-Borges et al., 2017). Sensitisation to mites is frequent in AD, with IgE to mite allergen found in 95% of patients with AD, compared to 42% of asthmatics and 17% of controls (Scalabrin et al., 1999). Mite proteases have direct epithelial effects including the breaching of tight junctions and the stimulation of protease-activated receptors, the latter inducing pruritus, epithelial dysfunction and cytokine release (Miller, 2019). Dust mite allergens with proteolytic activity include Der p 1, a cysteine protease and Der p 3, p 6 and p 9, which are serine proteases (Matricardi et al., 2016; Reithofer & Jahn-Schmid, 2017). Der p 2 is unusual in that it has sequence homology with MD-2, which, when bound to lipopolysaccharide (LPS), acts as the ligand binding to TLR-4, the pattern recognition receptor for endotoxin (Trompette et al., 2009). Der p 2 may, therefore, be acting as an “auto-adjuvant.” Der p 10 is tropomyosin, a muscle protein present in other invertebrates (Reese et al., 1999) and responsible for the cross-reactivity between dust mites and shrimp (Wong et al., 2016). Der p 11, another muscle allergen, has the unusually high molecular weight of 100 kDa, has homology with the paramyosin proteins found in invertebrates, is present in dust mite bodies rather than faeces, and is the significant dust mite allergen in AD (Banerjee et al., 2015; Fernandez-Caldas, 2013). Der p 23, a fairly recently identified dust mite allergen, is a peritrophin from the mite gut and is present in the outer membrane of mite faeces. Although IgE directed against it is common (Weghofer et al., 2013), it represents only a relatively small percentage of the total anti-dust mite IgE (Mueller et al., 2016). The most recently identified dust mite allergen is Der f 24, discovered in 2015 after the sequencing of the *D. farinae* genome (Chan et al., 2015). However, the allergenicity and clinical implications of these allergens are unknown (Park et al., 2018).

Over the past decade, the increased availability of allergenic molecules for diagnosis, known as precision allergy molecular diagnostic applications has improved the management of allergic diseases. Using recombinant allergens in order to establish the molecular diagnosis has greatly improved allergen characterisation and the knowledge of pathomechanisms causing allergic diseases. Molecular allergology allows a good view of the risk associated with sensitisation. Multi-allergen tests containing micro-arrayed allergen

molecules have been shown to be useful for the identification of culprit allergens in AD and may improve the management of AD by allergen-specific immunotherapy (AIT), allergen avoidance and IgE-targeting therapies in a personalised medicine approach (Heffler et al., 2018; van Hage et al., 2017).

Aim of the study

To analyse the sensitisation profile to molecular components of dust mites allergens with the use of ALEX2 Allergy Explorer test and to compare these results with the severity of AD, the occurrence of asthma bronchiale and allergic rhinitis in 100 AD patients.

Methods

Patients and methods

In the period 2018–2020, 100 patients suffering from AD were examined. All these patients were examined in the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech republic. The diagnosis of AD was made with the Hanifin-Rajka criteria (Hanifin & Rajka, 1980). Exclusion criteria were systemic therapy (cyclosporin, systemic corticoids, biological therapy), pregnancy, breastfeeding. Patients with AD having other systemic diseases were excluded from the study as well. Complete dermatological and allergological examination was performed in patients included in the study. This study was approved by Ethics committee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic.

Dermatological examination

Complete dermatological examination was performed in patients included in the study. Severity of AD was scored in agreement with SCORAD, with the assessment of topography items (affected skin area), intensity criteria and subjective parameters (European Task Force on Atopic Dermatitis, 1993).

Examination of specific IgE to allergen reagents

The serum level of the sIgE was measured by the components resolved diagnostic micro-array-based sIgE detection assay ImmunoCAP ALEX2 Allergy Explorer (Matricardi et al., 2016; Reithofer & Jahn-Schmid, 2017). It is based on a state-of-the-art proprietary nano-bead technology. The ALEX2 Allergy Explorer measuring range for specific IgE is 0.3–50 kU_A/L (quantitative) and for total IgE is 1–2500 kU/L (semiquantitative). The sample requirement is 100 µL serum or plasma. The results are expressed as Class 0 (<0.3kU_A/L) – negative, Class 1 (0.3 > 1 kU_A/L) – low positivity, Class 2 (1 > 5 kU_A/L) – moderate positivity, Class 3 (5 > 15 kU_A/L) – high positivity and Class 4 (≥15 kU_A/L) – very high positivity. The ALEX2 Allergy Explorer is commercially available, having attained CE certification, which assures that the quality of the assay, regarding the limit of detection, precision and repeatability as well as specificity and linearity, is in line with *in vitro* diagnostic features. There is no significant interference from high

total IgE, haemoglobin, bilirubin or triglycerides (Heffler et al., 2018; van Hage et al., 2017).

The review of allergenic molecules of mites according to the www.allergen.org is recorded in [Table 1](#). Molecular components examined in ALEX2 Allergy Explorer are shown extra bold*.

Statistical analysis

We evaluated with Fisher's Exact test the relation between the results of specific IgE to molecular components of mites and the occurrence of mild, moderate and severe forms of AD, in subgroup of patients suffering from asthma bronchiale and allergic rhinitis. The significance level is chosen at 5%. We used statistical software: NCSS 2021 Statistical Software (2021). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.

Results

Characteristic of patients

A total of AD patients were included in the study (49 men and 51 women with the average age 40.6 years: min. age 14 years, max. age 67 years and with the average SCORAD 39 points, SD 13.5 points). The mild form of AD was recorded in 14 patients (14%), moderate form in 61 patients (61%), severe form in 25 patients (25%); 55 patients (55%) suffer from bronchial asthma and 74 patients (74%) suffer from allergic rhinitis.

The detailed overview of molecular components of mites allergens according to the frequency of sensitisation in classes 0, 1, 2, 3, 4 in 100 AD patients (= 100%) is recorded in [Table 2](#). The highest sensitisation rate was confirmed to molecular components Der f 2 and Der p 2 – very high level of specific IgE (Class 4) reached 40% of patients. Sensitisation to molecular component Der p 23 was recorded in 36% of patients, very high level of specific IgE (Class 4) reached 23% of patients. The lowest sensitisation rate was confirmed to Der p 10 in 4% of patients and to Der p 11 in 3% of patients.

The relation between the results of specific IgE to molecular components of mites and the occurrence of mild, moderate and severe form of AD, in subgroup of patients suffering from asthma bronchiale and allergic rhinitis was evaluated with Fisher's Exact test. In [Table 3](#), we show the relative frequencies of sensitisation in mild, moderate and severe forms of AD and in subgroup of patients suffering from asthma bronchiale and allergic rhinitis. The sensitisation to Der f 2 has been shown to be significantly higher in patients with severe AD (in 64% of patients with severe form) as well as in patients with bronchial asthma and allergic rhinitis. In patients with severe AD, we demonstrated a higher incidence of sensitisation in all monitored components of mites in contrast to patients with mild form. Regarding the difference in sensitisation in the subgroup of asthmatic patients, we demonstrated a higher incidence of sensitisation in all monitored components, except for the Der p 10. Regarding the difference in sensitisation in the subgroup of patients suffering from allergic rhinitis, we demonstrated also a higher incidence of sensitisation in all monitored components, except for the Der p 10 and Blo t 10.

Table 1. Review of allergenic molecules of mites (www.allerge.org).

<i>Dermatophagoides farinae</i> (American house dust mite)		<i>Dermatophagoides pteronyssinus</i> (European house dust mite)			
Der f 1*	Cysteine protease	Der p 1*	Cysteine protease		
Der f 2*	NPC2 family	Der p 2*	NPC2 family		
Der f 3	Trypsin	Der p 3	Trypsin		
Der f 4	Alpha-amylase	Der p 4	Alpha-amylase		
Der f 5	Low molecular weight IgE binding protein	Der p 5*			
Der f 6	Chymotrypsin	Der p 6	Chymotrypsin		
Der f 7	Bactericidal permeability-increasing like protein	Der p 7*	Bactericidal permeability-increasing like protein		
Der f 8	Glutathione S-transferase	Der p 8	Glutathione S-transferase		
Der f 10	Tropomyosin	Der p 9	Collagenolytic serine protease		
Der f 11	Paramyosin	Der p 10*	Tropomyosin		
Der f 13	Fatty acid-binding protein	Der p 11*	Paramyosin		
Der f 14	Apolipophorin	Der p 13	Cytosolic Fatty Acid-Binding Protein		
Der f 15	Chitinase	Der p 14	Apolipophorin		
Der f 16	Gelsolin/villin	Der p 15	Chitinase-like protein		
Der f 17	Calcium binding protein	Der p 18	Chitin-binding protein		
Der f 18	Chitin-binding protein	Der p 20*	Arginine kinase		
Der f 20	Arginine kinase	Der p 21*			
Der f 21		Der p 23*	Peritrophin-like protein domain (PF01607)		
Der f 22		Der p 24	biquinol-cytochrome c reductase binding protein		
Der f 23	Peritrophin-like protein	Der p 25	Triosphosphate isomerase		
Der f 24	Ubiquinol-cytochrome c reductase binding protein homologue	Der p 26	Myosin light chain		
Der f 25	Triosephosphate isomerase	Der p 28	Heat Shock Protein 70		
Der f 26	Myosin alkali light chain	Der p 29	Cyclophilin		
Der f 27	Serpin	Der p 30	Ferritin		
Der f 28	Heat Shock Protein	Der p 31	Cofilin		
Der f 29	Peptidyl-prolyl cis-trans isomerase (cyclophilin)	Der p 32	Inorganic pyrophosphatase		
Der f 30	Ferritin	Der p 33	Alpha-tubulin		
Der f 31	Cofilin	Der p 36			
Der f 32	Secreted inorganic pyrophosphatase	Der p 37	Peritrophin-A domain containing protein		
Der f 33	alpha-tubulin	Der p 38	Bacteriolytic enzyme		
Der f 34	enamine/imine deaminase	Der p 39	Troponin C		
Der f 35					
Der f 36					
Der f 37	Chitin-binding protein				
Der f 38	Bacteriolytic enzyme				
<i>Blomia tropicalis</i> (Storage mite)		<i>Lepidoglyphus destructor</i> (Storage mite)		<i>Tyrophagus putrescentiae</i> (Storage mite)	
Blo t 1	Cysteine protease	Lep d 2*	NPC2 family	Tyr p 1	Cystein protease
Blo t 2	ML-domain protein	Lep d 5		Tyr p 2*	NPC2 family

(Continued)

Table 1. Continued.

<i>Dermatophagoides farinae</i> (American house dust mite)		<i>Dermatophagoides pteronyssinus</i> (European house dust mite)			
Blo t 3	Trypsin	Lep d 7	Bactericidal permeability-increasing like protein	Tyr p 3	Trypsin
Blo t 4	Alpha-amylase	Lep d 10	Tropomyosin	Tyr p 7	Bactericidal permeability-increasing protein
Blo t 5*		Lep d 13	Fatty acid-binding protein	Tyr p 8	Glutathione S-transferase
Blo t 6	Chymotrypsin			Tyr p 10	Tropomyosin
Blo t 7	Bactericidal permeability-increasing like protein			Tyr p 11	Paramyosin
Blo t 8	Glutathione S-transferase			Tyr p 13	Fatty acid-binding protein
Blo t 10*	Tropomyosin			Tyr p 20	Arginine Kinase
Blo t 11	Paramyosin			Tyr p 20	
Blo t 12				Tyr p 28	Heat shock protein
Blo t 13	Fatty acid-binding protein			Tyr p 32	Secreted inorganic pyrophosphatase
Blo t 19	Antimicrobial peptide homologue			Tyr p 34	Troponin C
Blo t 21*					
Glycyphagus domesticus (Storage mite)				Tyr p 35	Aldehyde dehydrogenase
Gly d 2*	Storage mite			Aca s *	Storage mite

Molecular components examined in ALEX2 Allergy Explorer are shown extra bold*.

Discussion

The purpose of our study is to evaluate the sensitisation to molecular components of mites in AD patients. Our study confirms that there are differences in mite sensitisation in AD patients with mild, moderate and severe forms and in subgroup of patients with respiratory allergy symptoms. Our results are in agreement with previous observations that Der f 2 is the most significant allergen from *Dermatophagoides farinae* (Becker et al., 2016; Čelakovská et al., 2020a; Čelakovská et al., 2020b; Čelakovská et al., 2021; Darsow et al., 2004; González-Pérez et al., 2021; Jacquet, 2011; Jeong et al., 2015; Panzner et al., 2018; Park et al., 2018; Ro et al., 2013).

Published sensitisation pattern to component allergens of mites is not consistent.

Although the HDM allergens are present in the mite bodies, the main allergenic sources are the mite faeces which, with a diameter higher than 10 µm (Becker et al., 2016), can be easily inhaled into the airways and consequently be entered deep into the lungs, so mite allergens associated with faecal particles (Der p 1, Der p 2, Der p 5, Der p 21, Der p 23) are more frequently recognised by patients with respiratory allergy (Becker et al., 2016; Resch et al., 2015; Weghofer et al., 2008). Whereas the full composition of mite faeces remains to be determined, we can easily speculate that mite faeces contain not only the identified HDM allergens but also numerous non-

Table 2. The detailed overview of molecular components of mites allergens according to the frequency of sensitisation in classes 0, 1, 2, 3, 4 in 100 AD patients (=100%).

The number of patients (%) according to the level of specific IgE in classes 0–4 – 100 patients include in the study = 100%					
	Sensitisation confirmed in patients (%)	Class 1 (%)	Class 2 (%)	Class 3 (%)	Class 4 (%)
Der f 2	45	1	2	2	40
Der p 2	44	0	2	2	40
Lep d 2	41	4	9	18	10
Der p 23	36	1	7	5	23
Der p 1	34	2	7	7	18
Gly d 2	33	9	8	4	12
Der f 1	31	3	5	12	11
Der p 5	26	8	3	1	14
Aca s	26	5	7	8	6
Tyr p	25	5	7	8	5
Der p 7	24	6	12	3	3
Der p 20	23	2	2	1	18
Der p 21	15	0	1	2	12
Blo t 5	12	3	3	3	3
Tyr p 2	11	4	6	1	0
Blo t 10	8	4	0	1	3
Blo t 21	5	2	3	0	0
Der p 10	4	0	0	1	3
Der p 11	3	1	1	0	1

Class 0 (< 0.3 kU_A/L) – negative; Class 1 (0.3 > 1 kU_A/L) – low positivity; Class 2 (1 > 5 kU_A/L) – moderate positivity; Class 3 (5 > 15 kU_A/L) – high positivity; Class 4 (≥ 15 kU_A/L) – very high positivity

allergenic mite proteins and macromolecules. On the other hand, Der p 11 and Der p 18 are more frequently recognised by IgE antibodies from patients with AD (Banerjee et al., 2015). Sensitisation to Der p 11 and Der p 18 may reflect the fact that the eczematous skin allows easy penetration of allergens even with molecular weight as high as 100,000. Der p 11 is a protein of 874 amino acids with a deduced molecular mass of ~103 kDa, and it shows high sequence identities (>85% sequence identity) with paramyosins from HDM, itchy mites and tropical mites (Banerjee et al., 2015). However, in our study, the sensitisation to Der p 11 was low; we recorded the sensitisation to Der p 11 in 1 patient (1%) with moderate form of AD and in 2 patients (2%) with severe form of AD.

Some studies show that the Der p 23-specific IgE levels of the tested patients were comparable to the IgE levels to Der p 1 and Der p 2. Because of its high frequency of IgE recognition and allergenic activity, Der p 23 must be considered as an essential component for the diagnosis and specific immunotherapy of HDM allergy (Becker et al., 2016; González-Pérez et al., 2021; Weghofer et al., 2008; Weghofer et al., 2013). In our study, we observe that sensitisation to Der p 23 increases from mild form (in 21.4% of patients) to moderate form (in 36.1% of patients) and to severe form (in 44.0% of patients), but the difference is not significant.

Patients with more severe forms of AD recognise a greater proportion of the allergen profile (including HDM allergens) in comparison to patients with moderate forms (Bousquet et al., 2015; Gaitanis et al., 2012; Lagier et al., 1995; Mittermann et al., 2016). It can be explained by the fact that the severity of AD may depend on the number of different allergen molecules recognised (Resch et al., 2015). An explanation can be that patients with more severe AD and broader IgE recognition profiles may be more “atopic” than patients with moderate AD (Mittermann et al., 2016). Earlier studies have shown that patients with poly-sensitisation to airborne allergens differed

Table 3. The relation between the results of specific IgE to molecular components of mites and the occurrence of mild, moderate and severe form of AD, in subgroup of patients suffering from asthma bronchiale and allergic rhinitis (Fisher's Exact test, the significance level is chosen at 5%).

Molecular component	Protein family	Number of patients – severity of AD			Number of patients – asthma bronchiale		Number of patients – allergic rhinitis	
		14 Mild	61 Moderate	25 Severe	45 AB–	55 AB+	26 AR–	74 AR+
Der f 1+	Cysteine protease	3	18	10	9	22	4	27
		(21.4 %)	(29.5 %)	(40.0 %)	(20.0 %)	(40.0 %)	(15.4 %)	(36.5 %)
Der f 1–		11	43	15	36	33	22	47
		(78.6 %)	(70.9 %)	(60.0 %)	(80.0 %)	(60.0 %)	(84.6 %)	(63.5 %)
<i>p</i> -value		0.4733			0.0496*		0.0516	
Der f 2+	NPC2 family	3	26	16	15	30	7	38
		(21.4%)	(42.6%)	(64.0%)	(33.3 %)	(54.5 %)	(26.9 %)	(51.3 %)
Der f 2–		11	35	9	30	25	19	36
		(78.6%)	(57.4%)	(36.0%)	(66.7 %)	(45.5%)	(73.1 %)	(48.6 %)
<i>p</i> -value		0.0366			0.0438*		0.0397	
Der p 1+	Cysteine protease	4	19	11	11	23	4	30
		(28.6%)	(31.2%)	(44.0%)	(24.4 %)	(41.8 %)	(15.4 %)	(40.5 %)
Der p 1–		10	42	66	34	32	22	44
		(71.4%)	(68.8%)	(56.0%)	(75.6 %)	(58.2 %)	(84.6 %)	(59.5 %)
<i>p</i> -value		0.4919			0.0901		0.0291	
Der p 2+	NPC2 family	1	26	15	15	29	7	37
		(21.4%)	(42.6%)	(60.0%)	(33.3 %)	(52.7 %)	(26.9 %)	(50.0%)
Der p 2–		11	35	56	30	26	19	37
		(78.6%)	(57.4%)	(40.0%)	(66.7 %)	(47.3 %)	(73.1 %)	(50.0 %)
<i>p</i> -value		0.0711			0.0687		0.0651	
Der p 5+	Low molecular weight IgE binding protein	1	14	11	7	19	3	23
		(7.1%)	(22.9%)	(44.0%)	(15.6 %)	(34.5 %)	(11.5 %)	(31.1 %)
Der p 5–		13	47	14	38	36	23	51
		(92.8%)	(77.1%)	(56.0%)	(84.4 %)	(65.5 %)	(88.5 %)	(68.9 %)
<i>p</i> -value		0.0336			0.0397		0.0686	
Der p 7+	Bactericidal permeability-increasing like protein	1	13	10	4	20	3	21
		(7.1%)	(21.3%)	(40.0%)	(8.9 %)	(36.4 %)	(11.5 %)	(28.4 %)
Der p 7–		13	48	15	41	35	23	53
		(92.9%)	(78.7%)	(60.0%)	(91.1 %)	(63.6 %)	(88.5 %)	(71.6%)
<i>p</i> -value		0.0671			0.0019		0.1109	
Der p 10+	Tropomyosin	1	1	2	2	2	1	3
		(7.1%)	(1.6 %)	(8.0%)	(4.4 %)	(3.6 %)	(3.8 %)	(4.1 %)

(Continued)

Table 3. Continued.

Molecular component	Protein family	Number of patients – severity of AD			Number of patients – asthma bronchiale		Number of patients – allergic rhinitis	
		14 Mild	61 Moderate	25 Severe	45 AB–	55 AB+	26 AR–	74 AR+
Der p 10–	Paramyosin	13 (92.8%)	60 (98.4%)	23 (92.0%)	43 (95.6 %)	53 (96.4 %)	25 (96.2 %)	71 (95.9 %)
<i>p</i> -value		0.3263			1.000		1.000	
Der p 11+		0 (0.0%)	1 (1.6%)	2 (8.0%)	0 (0 %)	3 (5.5 %)	0 (0 %)	3 (4.1 %)
Der p 11–	Arginine kinase	14 (100.0%)	60 (98.4%)	23 (92.0%)	45 (100.0 %)	52 (94.5%)	26 (100.0 %)	71 (95.9 %)
<i>p</i> -value		0.2460			0.2500		0.5657	
Der p 20+		1 (7.1%)	10 (16.4%)	12 (48.0%)	8 (17.8 %)	15 (27.3 %)	3 (11.5 %)	20 (27.0 %)
Der p 20–	No information found	13 (92.9%)	51 (83.6%)	13 (52.0%)	37 (82.2 %)	40 (72.7%)	23 (88.5 %)	54 (73.0 %)
<i>p</i> -value		0.0034			0.3412		0.1740	
Der p 21+		1 (7.1%)	7 (11.5%)	7 (28.0 %)	3 (6.7 %)	12 (21.8 %)	2 (7.7 %)	13 (17.6 %)
Der p 21–	Peritrophin-like protein domain	13 (92.9%)	54 (88.5%)	18 (72.0%)	42 (93.3 %)	43 (78.2 %)	24 (92.3 %)	61 (82.4 %)
<i>p</i> -value		0.1166			0.0482		0.3416	
Der p 23+		3 (21.4 %)	22 (36.1 %)	11 (44.0 %)	10 (22.2 %)	26 (47.3 %)	5 (19.2 %)	31 (41.9 %)
Der p 23–	Storage mite	11 (78.6 %)	39 (63.9 %)	14 (56.0 %)	35 (78.8 %)	29 (52.7 %)	21 (80.8 %)	43 (58.1 %)
<i>p</i> -value		0.3978			0.0121		0.0564	
Aca s+		2 (14.3%)	11 (18.0 %)	13 (52.0%)	8 (17.8 %)	18 (32.7 %)	5 (19.2 %)	21 (28.4 %)
Acas–	Mites group 5, storage mite	12 (85.7%)	50 (82.0%)	12 (48.0%)	37 (82.2 %)	37 (67.3 %)	21 (80.8 %)	53 (71.6 %)
<i>p</i> -value		0.0046			0.1112		0.4425	
Blo t 5+		1 (7.1%)	4 (6.6%)	7 (28.0%)	3 (6.7 %)	9 (16.4 %)	2 (7.7 %)	10 (13.5 %)
Blo t 5–	Tropomyosin storage mite	13 (92.9 %)	57 (93.4%)	18 (72.0%)	42 (93.3 %)	46 (83.6 %)	24 (92.3 %)	64 (86.5 %)
<i>p</i> -value		0.0264			0.2162		0.7264	
Blo t 10+		1 (7.1%)	3 (4.9%)	4 (16.0%)	3 (6.7 %)	5 (9.1 %)	2 (7.7 %)	6 (8.1 %)

Blo t 10–		Der f 1+ 13 (92.9%)	Cysteine	21 (84.0%)	42 (93.3 %)	50 (90.9 %)	24 (92.3 %)	68 (91.9 %)
<i>p</i> -value		0.2241			0.7269		1.000	
Blo t 21+	Storage mite	0 (0 %)	58 (95.1%)	21 (84.0%)	42 (93.3 %)	50 (90.9 %)	24 (92.3 %)	
			68 (91.9 %)					
<i>p</i> -value		0.2241				0.7269		1.000
Blo t 21+	Storage mite	0 (0 %)	1 (1.6%)	4 (16.0%)	0 (0 %)	5 (9.1 %)	0 (0 %)	5 (6.8 %)
Blo t 21–		14 (100.0%)	60 (98.4 %)	21 (84.0%)	45 (100.0 %)	50 (90.9 %)	26 (100.0 %)	69 (93.2 %)
<i>p</i> -value		0.0234			0.0624		0.3228	
Gly d 2+	NPC2 family	2 (14.3 %)	18 (29.5 %)	13 (52.0 %)	11 (24.4 %)	22 (40.0 %)	5 (19.2 %)	28 (37.8 %)
Gly d 2–		12 (85.7%)	43 (70.5%)	12 (48.0%)	34 (75.6 %)	33 (60.0 %)	21 (80.8 %)	46 (62.2 %)
<i>p</i> -value		0.0436			0.1349		0.0950	
Lep d 2+	NPC2 family	2 (14.3%)	24 (39.3%)	15 (60.0%)	14 (31.1 %)	27 (49.1 %)	7 (26.9 %)	34 (45.9 %)
Lep d 2–		12 (85.7%)	37 (60.7%)	10 (40.0%)	31 (68.9 %)	28 (50.9 %)	19 (73.1 %)	40 (54.1 %)
<i>p</i> -value		0.0204			0.1016		0.1081	
Tyr p+	Storage mite	2 (14.3%)	10 (16.4%)	13 (52.0%)	7 (15.6 %)	18 (32.7 %)	4 (15.4 %)	21 (26.4 %)
Tyr p–		12 (85.7%)	51 (83.6%)	12 (48.0%)	38 (84.4 %)	37 (67.3 %)	22 (84.6 %)	53 (71.6 %)
<i>p</i> -value		0.0023			0.0638		0.2920	
Tyr p 2+	NPC2 family	1 (7.1%)	4 (6.6%)	6 (24.0%)	3 (6.7 %)	8 (14.6 %)	2 (7.7 %)	9 (12.2 %)
Tyr p 2–		13 (92.9%)	57 (93.4%)	19 (76.0%)	42 (93.3 %)	47 (85.4 %)	24 (92.3 %)	65 (87.8 %)
<i>p</i> -value		0.0735			0.3364		0.7233	

We show the number of patients in % (14 patients with mild form = 100%, 61 patients with moderate form = 100%, 25 patients with severe form = 100%, 45 patients without AB (AB–) = 100%, 55 patients with AB (AB+) = 100%, 26 patients without AR (AR–)=100%, 74 patients with AR (AR+) = 100%). The significant relation is recorded extra bold *.

from monosensitised patients by their production of IL-4 and Th2-driven cytokines (Lagier et al., 1995).

According to our results, in patients suffering from severe forms of AD, asthma bronchiale and allergic rhinitis the important roles play molecular components from NPC2 family (Der f 2, Lep d 2) and arginine kinase (Der p 20). NPC2 belongs to the ML (MD-2-related lipid-recognition) protein family (155 members), which includes the Toll-like receptor co-factors, MD-1 and MD-2, and seven major HDM allergens of unknown function, including Der p 2 and Der f 2 (Pathak & Helm, 2010).

Another reason for the different HDM sensitisation is the geographical location. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* can be found all over the world, while *Blomia tropicalis* is the predominant mite species in tropical and subtropical regions, and in some areas, it coexists with *Dermatophagoides pteronyssinus* (Banerjee et al., 2015; Carvalho Kdos et al., 2013; Mueller, Gosavi, et al., 2010; Santos da Silva et al., 2017). Group 5 allergens from HDMs elicit strong IgE antibody binding in mite-allergic patients. The significantly higher sensitisation to Blo t 5, Blo t 21 and Der p 5 in patients suffering from a severe form of AD was confirmed also in our study. In addition, molecular component Der p 5 is also significantly higher in patients suffering from bronchial asthma. It was reported that Der p 5 activated human airway-derived epithelial cells via a protease-independent mechanism. Recently, crystallographic studies revealed that Der p 7 (Bactericidal permeability-increasing like protein) was distantly related to hydrophobic ligand-binding proteins of the human innate immune system. It was suggested that Der p 7 may co-opt the innate immune response into promoting allergenicity through the binding and delivery of hydrophobic ligands, as has been demonstrated for the mite allergen Der p 2 (Mueller, Edwards, et al., 2010). The results of our study show, that in AD patients who suffer from bronchial asthma, the significantly higher sensitisation to Der p 5 and Der p 7 was confirmed. Curin shows that Der p 5 and Der p 21 contain a major conformational IgE epitope-containing area located on similar portions of their structure, but they lack relevant IgE cross-reactivity. These data are important for the development of modern allergy vaccines based on defined molecules for allergen-specific immunotherapy of HDM allergy (Curin et al., 2018).

Wang evaluated the role of specific IgE against Der p 1 and Der p 2 in Chinese patients with HDM allergy. Der p 1 and Der p 2 are the dominant allergens in patients with HDM allergy in China. The relative importance of Der p 1 and Der p 2 changes with age, in favour of Der p 2. Overall, specific IgE titres were positively associated with the number of organs affected (including the eyes, nose, lungs and skin) (Wang et al., 2015). Kowal evaluated the pattern of IgE sensitisation to three major *Dermatophagoides pteronyssinus* allergens among patients from North Eastern Poland suffering from HDM-allergic rhinitis and/or allergic asthma. The frequency of positive results for individual allergens depended on clinical manifestations and the level of IgE to the whole *Dermatophagoides pteronyssinus* extract. In HDM- patients with allergic asthma, reactivity to nDer p 1 and rDer p 23 was detected more frequently than in HDM- patients with allergic rhinitis (Kowal et al., 2020). Vidal investigated the association between the sensitisation profile of adolescent and adult HDM-allergic patients and asthma in a region with high rates of exposure to HDM. Significantly higher concentrations of specific IgE to Der p 1, Der p 2 and to *Lepidoglyphus destructor* were observed in patients with

asthma than in those without asthma. *Lepidoglyphus destructor* may be a relevant allergen in high-exposure areas. Dual sensitisation (i.e. IgE to both Der p 1 and Der p 2) may help to identify HDM-allergic patients who are at risk of asthma (Vidal et al., 2016). Actual data on allergen avoidance have shown that, with some exception, it is generally not effective in controlling AD in children; a recent meta-analysis was not in favour of HDM avoidance strategies in established AD. High-quality long-term trials of single, easy to apply house dust mite reduction or avoidance measures are needed (Cadario et al., 2007; Pajno et al., 2007). The majority of clinical trials looking into AIT as a potential treatment for AD were carried out with adult patients; the efficacy of AIT as a treatment modality in children with AD has been the subject of few studies, with conflicting data. A well-performed study showed significant improvement of AD outcomes only in the mild/moderate group and not in the severe group (Tam et al., 2016; Werfel et al., 2006). Molecular diagnosis can provide a basis for further research, exploring the genetic and environmental factors that could be responsible for different severity of AD phenotypes leading to improved disease management following the principle of personalised medicine approach. According to our results, the specific immunotherapy should focus on the components of NPC2 family and Der p 23 (Peritrophin-like protein domain).

Conclusion

Our study confirms that there are differences in mite sensitisation in patients with a different form of AD and in patients with respiratory allergy symptoms. In patients with severe AD, we demonstrated a higher incidence of sensitisation in all monitored components of mites in contrast to patients with mild form. The central role in atopic march may play the molecular component Der f 2 and Der p 23. The results of our study confirm the important role of Der p 5 and Der p 7 in subgroup of patients suffering from bronchial asthma. Our results may be helpful in planning hyposensitisation, treatment and immunotherapy.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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