Sensitization to Molecular Components in 104 Atopic Dermatitis Patients in Relation to Subgroups of Patients Suffering from Bronchial Asthma and Allergic Rhinitis

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ABSTRACT

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease. The progression from AD to bronchial asthma (AB) and allergic rhinitis (AR) is called atopic march. The aim of this study was to evaluate the difference in the sensitization to molecular components in patients suffering from AD in relation to subgroups of patients with AR and AB.

Material and Methods: The complete dermatological and allergological examinations were performed. Specific IgE antibodies against 112 molecular components were measured with the multiplex ImmnoCAP ISAC test.

Results: Altogether 104 atopic dermatitis patients (50 men, 54 women) at the average age 40.1 years were examined. The sensitization to molecular components was confirmed in 93.3% of patients. The sensitization to components of mites, grasses, trees, animals, moulds, and shrimps was significantly more frequent in patients with severe form of AD and the sensitization to components of grasses, trees, and moulds was significantly higher in subgroup of patients with AB. In subgroup of patients suffering from AR the higher occurrence of pollen-derived and pollen-food derived PR-10 proteins, grasses, mites, and animals was observed also.

Conclusions: We have confirmed the significant differences in the sensitization to molecular components in patients suffering from severe form of AD, and in subgroups of patients suffering from AB and AR. These molecular components may play the important role in the consecutive development of different allergy pathologies called atopic march.

KEYWORDS

molecular components; multiplex ISAC testing; severity of atopic dermatitis; bronchial asthma; allergic rhinitis; atopic march

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INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease. The pathogenesis of AD involves susceptibility genes, immune dysregulation, and disrupted epidermal barrier function resulting in increased transepidermal water loss (TEWL), permeation of irritants, microbes and aeroallergens (1–3). The immune response is polarized towards innate immunity cells, such as dendritic cells, innate lymphoid cells type 2 (ILC-2), mast cells, basophilic granulocytes, and eosinophilic granulocytes. The direct contact of skin with allergens could trigger signals to initiate Th2 allergic response. A typical manifestation of allergic inflammation is the production of IgE antibodies directed against causative allergens (4, 5). A progression from AD to allergic rhinitis (AR) and bronchial asthma (AB) may develop in the first several years of life. This process is a phenomenon called atopic march (6). Positive correlations have been demonstrated between the severity of AD and the risk of development bronchial asthma, and allergic rhinitis (1). However, the exact mechanism explaining the atopic march remains to be elucidated. Emerging data suggest that epithelial cell-derived cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 may drive the progression from AD to bronchial asthma and food allergy (1). Various allergens may cause exacerbation of eczematous skin lesions in atopic dermatitis. The main allergenic sources are food, moulds, trees, weeds, grasses, mites, and animals (7). Specific IgE sensitization to food and aeroallergens, especially to house dust mites, pollen-derived and plant-derived food allergens has been described in adult AD patients (8).

Diagnostic tests of allergic diseases such as *in-vivo* skin prick tests or *in-vitro* measurement of specific IgE, and basophil activation test, are based on allergens derived from natural sources (extracts). Each allergen source is a very complex mixture of allergenic and non-allergenic proteins. This methodology has its limitations. Allergic extracts are incapable to differentiate between primary sensitization and immunological cross-reactivity (9–11). Progress in laboratory diagnostics of IgE-mediated allergy was made by the introduction of component-resolved diagnosis (CRD). The molecularly defined allergens (components) are used in a singleplex test or a multiplex allergen microarray assay. The main goal of CRD is to distinguish between the mainly species-specific components and the cross-reactive allergen molecules. It is evident that CRD enhances the specificity of IgE-diagnosis in polysensitized respiratory allergies (12), and can be also applied in food allergies (13) and atopic dermatitis (13, 14) and in addition, may reveal unexplained anaphylaxis (10).

The aim of this study was to evaluate the sensitization to molecular components in relation to severity of AD and to determine whether there are some differences between the sensitization profiles in subgroups of patients suffering from bronchial asthma and allergic rhinitis. To identify the sensitization and co-sensitization to species-specific and cross-reacting allergen components we used a commercially available microarray immunoassay ImmunoCAP ISAC. It is a complex assay for simultaneous determination of allergen specific IgE (sIgE) against 112 molecular components (purified natural and recombinant) originating for more than 50 sources (14, 15). The major advantage of ISAC is the comprehensive IgE pattern obtained with a minute amount of serum (16).

Only few reports demonstrate the sensitization to molecular components in atopic dermatitis patients and the relation of this sensitization to the severity of atopic dermatitis, and to the occurrence of bronchial asthma and allergic rhinitis (8, 17, 18). It should be emphasized that in this study we focused on the degree of sensitization only and not on its clinical relevance.

MATERIAL AND METHODS

PATIENTS

In the period 2018–2019, 104 patients suffering from atopic dermatitis were examined. All these patients were examined at the Department of Dermatology and Venereology, University Hospital Hradec Králové, Czech Republic. Complete dermatological and allergological examination was performed in all patients enrolled to this study. The diagnosis of atopic dermatitis was made using the Hanifin-Rajka criteria (19). Exclusion criteria were long term therapy with cyclosporin or systemic corticoids, pregnancy, breastfeeding. Patients with atopic dermatitis having other systemic diseases were excluded from the study as well. This study was approved by the Ethics Committee, University Hospital Hradec Králové, Czech Republic.

BRONCHIAL ASTHMA

The diagnosis of bronchial asthma (AB), was determined according to the guidelines of the Global Initiative for Asthma (GINA) at allergy outpatients clinic of the Institute of Clinical Immunology and Allergology, University Hospital Hradec Králové, Czech Republic.

ALLERGIC RHINITIS

The evaluation of allergic rhinitis (AR), was made according to the allergy testing and personal history.

SEVERITY OF ATOPIC DERMATITIS

Severity of atopic dermatitis was scored according to SCORAD index (Scoring of Atopic Dermatitis) with the assessment of topography items (affected skin area), intensity criteria and subjective parameters (20). The severity of atopic dermatitis was evaluated with SCORAD index as a mild form to 25 points, as a moderate form over 25 to 50 points, as a severe form over 50 points. The evaluation of the severity was calculated as the average SCORAD measured every 2 month during 1 last year (21).

EXAMINATION OF SPECIFIC IGE TO MOLECULAR COMPONENTS BY IMMUNOCAP ISAC TEST

Samples of blood were collected from the cubital vein. Blood serum was isolated by centrifugation and stored under –70 °C until analysis. Repeated thawing and freezing

were avoided. The levels of specific IgE in all patients were determined by the component-resolved diagnosis microarray-based sIgE detection assay ImmunoCAP ISAC sIgE 112 (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP ISAC sIgE 112 is a solid-phase semi-quantitative multiple immunoassay which enables to determine 112 different components from 51 allergen sources (22, 23). The molecular components are applied in triplicates (70 recombinant, 42 purified natural) to ensure the test reproducibility. The specific IgE values are measured in arbitrary units ISU-E (ISAC Standardized Units), measuring range of 0.3–100 ISU-E. The results of sIgE are presented semi-quantitatively in 4 classes: < 0.3 ISU-E negative, 0.3 > 0.9 ISU-E low positivity, 0.9 > 15 ISU-E moderate positivity, ≥ 15 ISU-E very high positivity (the level of specific IgE greater than 0.3 ISU-E was considered as positive) (14). The analysis was conducted according to the manufacturer's instruction.

STATISTICAL ANALYSIS

We analysed the data to determine whether the occurrence of sensitization to examined molecular components is in relation to the severity of atopic dermatitis. In addition, we assess if there are some differences in the sensitization to molecular components in the subgroups of patients suffering from bronchial asthma or allergic rhinitis. Relative frequencies of sensitization to the investigated molecular components were determined in all patients according to the severity of atopic dermatitis, bronchial asthma and allergic rhinitis. Pairs of these categories were enrolled in the contingency tables and the Chi-square independence test was performed. The significance level was set to 5%.

RESULTS

We examined 104 patients suffering from AD, 50 men and 54 women with the average age 40.1 years (s.d. 15.9) and with the average SCORAD index 39 points (s.d. 13.1). Mild form of AD was recorded in 13.5% of patients, moderate form of AD in 58.7% of patients and severe form of AD in 27.9% of patients. Subgroup of patients suffering from bronchial asthma or allergic rhinitis was recorded in 55.8% and 76.0%, respectively. The sensitization to at least one of the tested molecular components was confirmed in 93.3% of patients. No positive results to molecular components were obtained in 6.7% patients. The characteristics of the patients are summarised in Table 1. The results describing the sensitization patterns to tested components in all AD patients are listed below and shown in Table 2.

Tab. 1 The characteristics of patients.

Number of patients with AD	**104 patients (50 men, 54 women)**
age	average age 40.1 years (s.d. 15.9)
index SCORAD	average SCORAD 39 points (s.d. 13.1)
sensitization to allergen components	97 patients (93.3%)
mild form of AD	14 patients (13.5%)
moderate form of AD	61 patients (58.7%)
severe form of AD	29 patients (27.9%)
subgroups of patients:	
number of patients with AB	58 patients (55.8%)
number of patients with AR	79 patients (76.0%)

AD – atopic dermatitis, AB – bronchial asthma, AR – allergic rhinitis

In the whole group of patients, the highest sensitization rate was observed to pollen-derived components and *Betulaceae*-specific components. Timothy is present on the biochip in eight molecular components. Sensitization rate to rPhl p 1 (61.0%) was followed by nPhl p 4 (52.0%), rPhl p 5 (43.0%), rPhl p 6 (42.0%), rPhl p 2 (39.0%) and rPhl p 11 (20.0%). The sensitization rate to polcalcin rPhl p 7 and profilin rPhl p 12 was lower than 10.0%. The second most frequent sensitization was 57.0% to rBet v 1, which was followed by other *Betulaceae*-specific components, such as rCor a 1.0101 (45.0%) and rAln g 1 (43.0%). Sensitization to pollen-food derived PR-10 proteins was observed frequently as well; on the other hand, sensitization to profilin rBet v 2 and polcalcin rBet v 4 were observed rarely (< 10%). The sensitization rates to mite-specific molecules were observed more frequently in the group 2 (rDer p 2, 46.0% and rDer f 2, 45.0%) in comparison with group 1 (nDer p 1, 36.0% and nDer f 1, 34.0%). The sensitization to animal components was observed most frequently to cat allergen rFel d 1 (42.0%) and dog allergens rCan f 1 (39.0%) and rCan f 5 (26.0%), which were followed by the sensitization to animal lipocalins rFel d 4 (29.0%), rEqu c 1 (27.0%), nMus m 1 (20.0%), rCan f 2 (17.0%). The frequency of sensitization to individual components is shown in Table 2 and schematically illustrated in Figure related to Table 2.

SENSITIZATION TO THE MOLECULAR COMPONENTS IN RELATION TO THE SEVERITY OF ATOPIC DERMATITIS

All 104 patients were divided into three groups according to SCORAD index. We evaluated the relative frequency of positive reactions to molecular components in patients with mild, moderate and severe form of atopic dermatitis. Increased relative frequency of positive reactions ranging from mild to moderate to severe form of AD was confirmed for most molecular components. Positive results of specific IgE antibodies against 47 molecular components were presented in mild form of AD, and 105 components were recorded in moderate and severe form of AD.

In the severe form of AD (29 patients; 100%) the highest sensitization rate to grass-species specific component rPhl p 1 (timothy, beta-expansin) reached 72.4% of patients. The second most frequent sensitization rate to components of mites rDer f 2 and rDer p 2 (NPC2 family) was observed in 65.5% of patients with severe form of AD.

The relation between the occurrence of sensitization to some molecular components and the severity of AD was confirmed. The following molecular components were recorded significantly more frequently in patients with severe form of AD than with mild form of AD ($p < 0.05$).

Tab. 2 The list of molecular components according to positivity (relative frequency) in 104 patients with atopic dermatitis.

Allergen source	Molecular components	Protein groups	*No.* of patients (%)
Timothy grass	**rPhl p 1**	β-expansin	61.0
Birch	**rBet v 1**	PR-10 protein	57.0
Timothy grass	*nPhl p 4*	Berberine bridge enzyme	52.0
Bermuda grass	**nCyn d 1**	β-expansin	49.0
Apple	**rMal d 1**	PR-10 protein	47.0
House dust mite	**rDer p 2**	NPC2 family	46.0
Peach	**rPru p 1**	PR-10 protein	46.0
Hazel pollen	**rCor a 1.0101**	PR-10 protein	45.0
House dust mite	**rDer f 2**	NPC2 family	45.0
Timothy grass	**rPhl p 5**	Ribonucleases	43.0
Alder	**rAln g 1**	PR-10 protein	43.0
Timothy grass	*rPhl p 6*	Grass group 6	42.0
Cat	**rFel d 1**	Uteroglobin	42.0
Hazelnut	**rCor a 1.0401**	PR-10 protein	42.0
Timothy grass	*rPhl p 2*	Expansin	39.0
Dog	**rCan f 1**	Lipocalin	39.0
Peanut	**rAra h 8**	PR-10 protein	38.0
House dust mite	**nDer p 1**	Cysteine protease	36.0
House dust mite	**nDer f 1**	Cysteine protease	34.0
Soy	**rGly m 4**	PR-10 protein	32.0
Mugwort	**nArt v 1**	Defensin	29.0
Alternaria	**rAlt a 1**	Acidic glycoprotein	29.0
Cat	**rFel d 4**	Lipocalin	29.0
Horse	**rEqu c 1**	Lipocalin	27.0
Dog	**rCan f 5**	Arginine esterase, Prostatic kallikrein	26.0
Plane tree	**nPla a 2**	Polygalacturonase	24.0
Kiwifruit	**rAct d 8**	PR-10 protein	24.0
Shrimp	*nPen m 2*	Arginine kinase	22.0
Celery	**rApi g 1**	PR-10 protein	22.0
CCD	nMUXF3	Sugar epitope from bromelain	22.0
Aspergillus	*rAsp f 6*	Mn superoxide dismutase	21.0
Kiwifruit	**nAct d 2**	Thaumatin-like protein	21.0
Timothy grass	*rPhl p 11*	Ole e 1-related protein	20.0
Mouse	**nMus m 1**	Lipocalin	20.0
Olive pollen	**rOle e 9**	1.3-β-glucanase	17.0
Dog	*rCan f 2*	Lipocalin	17.0
Alternaria	*rAlt a 6*	Enolase	16.0
Japanese cedar	**nCry j 1**	Pectate lyase	15.0
Plantain	**rPla l 1**	Ole e 1-related protein	15.0
Cypress	**nCup a 1**	Pectate lyase	14.0
Olive pollen	**rOle e 1**	Common olive group 1	14.0
Annual mercury	*rMer a 1*	Profilin	13.0
Storage mite	*rLep d 2*	NPC2 family	13.0
Latex	*rHev b 8*	Profilin	13.0
Kiwifruit	**nAct d 1**	Cysteine protease	11.0
Wall pelitory	**rPar j 2**	Lipid transfer protein	10.0
Yellow jacket	**rVes v 5**	Antigen 5	10.0

CCD – cross-reactive carbohydrate determinants; major allergens are highlighted in bold (e.g. **rPhl p 1**), minor allergens are highlighted in italics (e.g. *rPhl p 6*) and cross-reactive components are illustrate in grey box (e.g. **rBet v 1**); molecular components with sensitization rate less than 10% are not mentioned

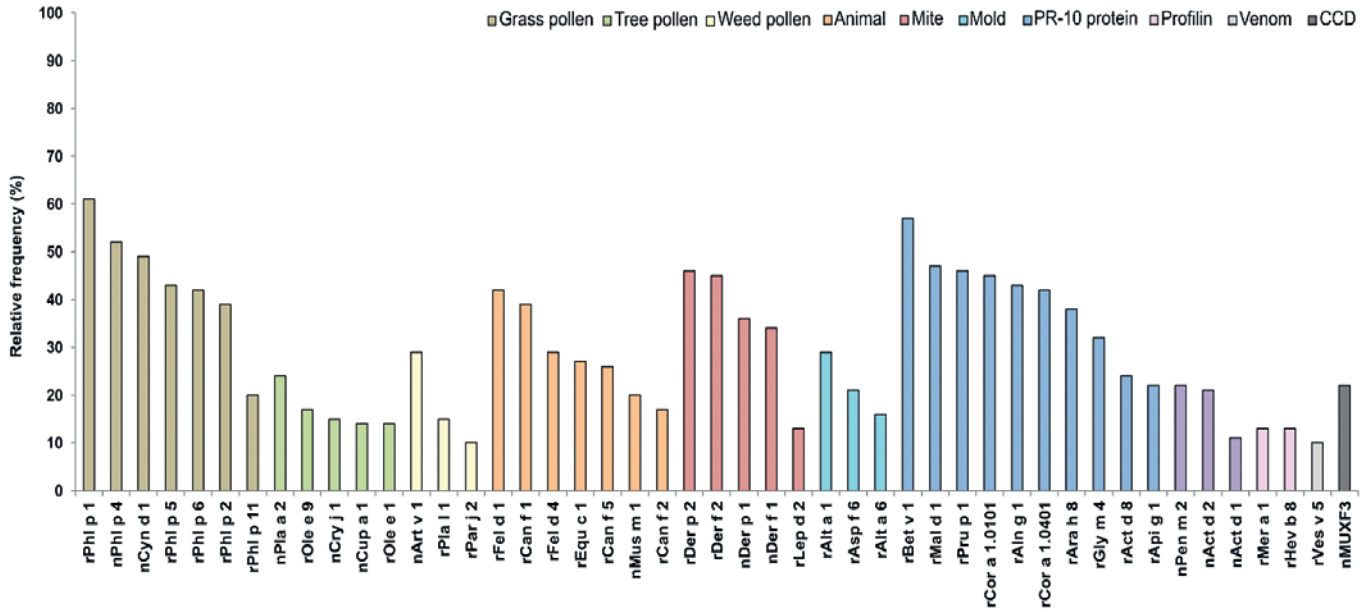

Fig. 1 related to Tab. 2: Sensitization rate to molecular components according to positivity (relative frequency) in 104 patients with atopic dermatitis. Molecular components are sorted by protein group and decreasing relative frequency in each group; molecular components with sensitization rate less than 10% are not mentioned.

High sensitization rate of positive reactions were reported against the major inhalant allergen components of grasses rPhl p 1 (timothy, beta-expansin); mites rDer f 2 and rDer p 2 (house dust mite, NPC2 family); animals rCan f 1 (dog, lipocalin), rCan f 5 (dog, arginine esterase), rFel d 1 (cat, uteroglobin), rFel d 4 (cat, lipocalin), rEqu c 1 (horse, lipocalin), nMus m 1 (mouse, lipocalin); and vegetables rApi g 1 (celery, PR-10 protein) in severe form of AD. The

Tab. 3 The list of molecular components according to positivity (relative frequency) in mild, moderate and severe form of AD – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	No. of patients in mild form of AD (%)	No. of patients in moderate form of AD (%)	No. of patients in severe form of AD (%)	p-value
		(14 patients = 100%)	(61 patients = 100%)	(29 patients = 100%)	
Timothy grass	**rPhl p 1**	4 (28.6%)	40 (65.6%)	21 (72.4%)	0.015
House dust mite	**rDer f 2**	2 (14.3%)	26 (42.6%)	19 (65.5%)	0.006
House dust mite	**rDer p 2**	2 (14.3%)	27 (44.3%)	19 (65.5%)	0.006
Cat	**rFel d 1**	2 (14.3%)	27 (44.3%)	17 (58.6%)	0.023
Cat	**rFel d 4**	0 (0.0%)	14 (23.0%)	16 (55.2%)	0.000
Dog	**rCan f 1**	0 (0.0%)	27 (44.3%)	13 (44.8%)	0.006
Dog	**rCan f 5**	1 (7.1%)	14 (23.0%)	12 (41.4%)	0.040
Horse	**rEqu c 1**	0 (0.0%)	14 (23.0%)	15 (51.7%)	0.001
Mouse	**nMus m 1**	0 (0.0%)	10 (16.4%)	11 (37.9%)	0.008
Olive	**rOle e 9**	0 (0.0%)	10 (16.4%)	9 (31.0%)	0.040
Birch	*rBet v 2*	0 (0.0%)	3 (4.9%)	6 (20.7%)	0.021
Alternaria	*rAlt a 6*	0 (0.0%)	7 (11.5%)	10 (34.5%)	0.005
Aspergillus	*rAsp f 6*	0 (0.0%)	11 (18.0%)	12 (41.4%)	0.004
Shrimp	*nPen m 2*	1 (7.1%)	7 (11.5%)	14 (48.3%)	0.000
Celery	**rApi g 1**	0 (0.0%)	12 (19.7%)	10 (34.5%)	0.031
Yellow jacket	**rVes v 5**	0 (0.0%)	3 (4.9%)	7 (24.1%)	0.006
Walnut	**nJug r 2**	0 (0.0%)	3 (4.9%)	6 (20.7%)	0.021
Egg white	**nGal d 2**	0 (0.0%)	0 (0.0%)	5 (17.2%)	0.001
Peanut	**rAra h 1**	0 (0.0%)	1 (1.6%)	4 (13.8%)	0.028
Wheat	**nTri a aA_TI**	0 (0.0%)	0 (0.0%)	3 (10.3%)	0.018

AD – atopic dermatitis; major allergens are highlighted in bold (e.g. **rPhl p 1**), minor allergens are highlighted in italics (e.g. *rAlt a 6*), and cross-reactive components are illustrate in grey box (e.g. **rApi g 1**)

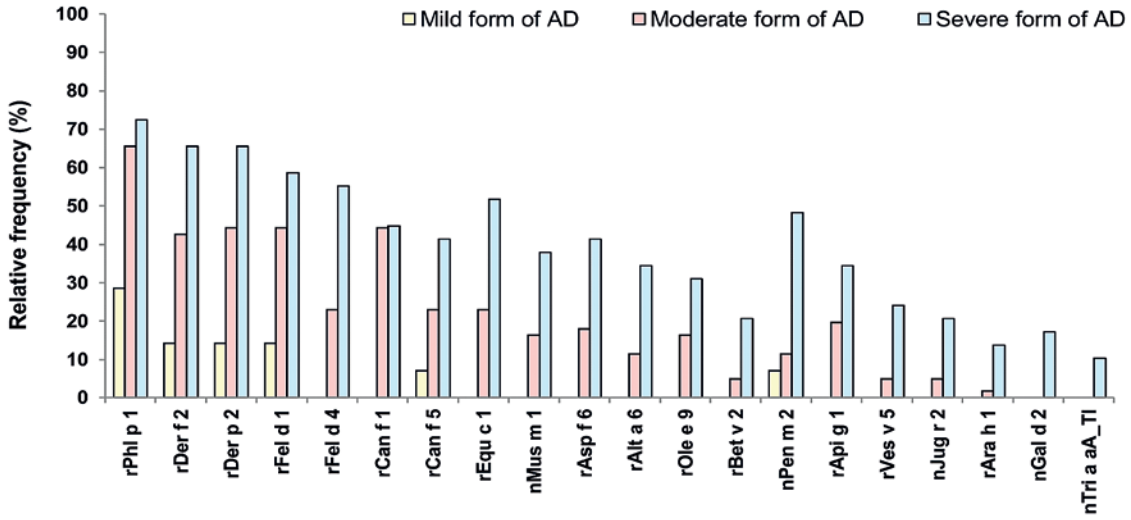

Fig. 2 related to Tab. 3: Sensitization to molecular components according to positivity (relative frequency) in mild, moderate and severe forms of AD – statistically significant difference (p-value < 0.05).

sensitization rate to minor allergen components of moulds rAlt a 6 (*Alternaria,* enolase), rAsp f 6 (*Aspergillus,* Mn superoxide dismutase), and crustaceans nPen m 2 (shrimp, arginine kinase), was also high. Furthermore, significant differences were confirmed, but with a lower frequency of positive cases, against to tree pollen allergens, such as major component of olive rOle e 9 (glucanase) and to minor component of birch rBet v 2 (profilin) in severe form of AD. Moreover, the lower frequency of positive cases were observed in the major food allergens, such as nTri a aA_TI (wheat, trypsin/α-amylase inhibitor), rAra h 1 (peanut, 7S globulin), nJug r 2 (walnut, 7S globulin), nGal d 2 (egg white, ovalbumin), and the major allergen of wasp rVes v 5 (yellow jacket, antigen 5). These differences (p-value < 0.05) are shown in Table 3 and schematically illustrated in Figure related to Table 3.

SENSITIZATION TO THE MOLECULAR COMPONENTS IN RELATION TO SUBGROUPS OF PATIENTS SUFFERING FROM ALLERGIC RHINITIS AND BRONCHIAL ASTHMA

We determined whether there are some differences between the sensitization to molecular components in relation to concomitant bronchial asthma or allergic rhinitis in all of 104 atopic dermatitis patients.

The occurrence of AB was recorded in 55.8% of patients. Following molecular components were observed significantly more frequently in patients with AB, such as a minor grass-specific component rPhl p 2 (timothy, expansin), and a major component of trees rOle e 9 (olive, glucanase). Sensitization rate to the minor component of mould rAlt a 6 (*Alternaria,* enolase) was also high. Moreover, lower frequency of positive case against the major allergen of wasp rVes v 5 (antigen 5) was noticed in patients with AB. Interestingly, the sensitization rate to polcalcin regarding rPhl p 7 (timothy) and rBet v 4 (birch) showed no positive results of sIgE in patients with AB. Surprisingly, the occurrence of the CCD component MUXF3 were observed more frequently in subgroup of patients with AB. These differences (p-value < 0.05) are shown in Table 4 and schematically illustrated in Figure related to Table 4.

The occurrence of AR was recorded in 76.0% of patients. These molecular components were observed significantly more frequently in patients with AR: major components of pollen-derived and pollen-food derived PR-10 proteins (Bet v 1 family), such as rBet v 1 (birch), rCor a 1.0101 (hazel), rMal d 1 (apple), rPru p 1 (peach), and rApi g 1 (celery). Sensitization rate to the major grass-specific components nCyn d 1 (bermuda grass, beta-expansin), rPhl p 5 (timothy, ribonuclease), and a minor component rPhl p 6 (timothy, grass group 6), and a major component of house dust mite rDer f 2 (NPC2 family) and lipocalins, such as rCan f 1 (dog), rFel d 4 (cat), rEqu c 1 (horse), nMus m 1 (mouse) was also high in patients with AR. The sensitization rate to nAct d 1 (kiwifruit, cysteine protease) was less frequent. These differences (p-value < 0.05) are shown in Table 5 and schematically illustrated in Figure related to Table 5.

DISCUSSION

Skin barrier abnormalities have been proposed to play an essential role in the initiation of atopic dermatitis in infancy (6). Epicutaneous allergens sensitization through an impaired skin barrier stimulates antigen-presenting cells and induces Th2 responses and consequent allergic manifestations. In a Th2-promoting environment, T-cell/B-cell interactions in regional lymph nodes lead to an excessive IgE switch (1). Simultaneous release of memory T cells into the circulation and their homing back to the skin can induce not only exacerbation of AD but also can initiate the atopic march. The progression of atopic disorders from AD in infants to allergic rhinitis and asthma in children is usually described as atopic march. The most important factor that precipitates atopic march is now considered an impaired epidermal barrier. Barrier disturbances result from genetic defects and early epicutaneous sensitization to food and aeroallergens may be enhanced by damage of the skin barrier function (6, 24). However, the exact

Tab. 4 The list of molecular components according to positivity (relative frequency) in subgroup of patients suffering from bronchial asthma – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	*No.* of patients without AB (%) (46 patients = 100%)	*No.* of patients with AB (%) (58 patients = 100%)	p-value
Timothy grass	*rPhl p 2*	13 (28.3%)	28 (48.3%)	0.038
Olive	**rOle e 9**	4 (8.7%)	15 (25.9%)	0.024
Alternaria	*rAlt a 6*	2 (4.3%)	15 (25.9%)	0.003
CCD	nMUXF3	6 (13.0%)	17 (29.3%)	0.047
Yellow jacket	**rVes v 5**	1 (2.2%)	9 (15.5%)	0.022
Birch	*rBet v 4*	4 (8.7%)	0 (0.0%)	0.022
Timothy grass	*rPhl p 7*	7 (15.2%)	0 (0.0%)	0.002

AB – bronchial asthma, CCD – cross-reactive carbohydrate determinants; major allergens are highlighted in bold (e.g. **rOle e 9**), minor allergens are highlighted in italics (e.g. *rAlt a 6*), and cross-reactive components are illustrate in grey box (e.g. *rBet v 4*)

mechanisms explaining the atopic march remain to be elucidated.

Progress in laboratory diagnostics of IgE-mediated allergies is the use of component-resolved diagnosis that implies determination of sIgE against purified native and recombinant components which are used in laboratory as singleplex or multiplex assays (25). There is currently no consensus on the use of multiplex microarray ImmunoCAP ISAC worldwide (26–28). Hatzler et al. (29) investigated the IgE response to grass-specific pollen allergens and determined that sensitization can start years before clinical disease onset through the process called "molecular spreading". There is some evidence (30, 31) that studies show a strong correlation between results of extract-based skin prick testing (SPT), multiplex microarray assay (ImmunoCAP ISAC, Phadia) and fluorescence enzyme immunoassays (UniCAP, Phadia) with excellent correlation especially in pollen allergens (32) and house dust mite allergens (33). Molecular allergy diagnosis may improve the risk evaluation, sorts out genuine from cross-reactive sensitizations, and finally, improves the accuracy of allergen immunotherapy indication. Currently, more than 130 molecular components are available for *in-vitro* sIgE testing which can be performed on singleplex or multiplex measurement platforms (e.g. for ALEX^2 more than 170 components) (5). In the WAO-ARIA-GA2LEN consensus document (5) molecular-based allergy diagnosis is recommended in the third line-diagnostic workup, if medical history and exact-based skin prick- and sIgE testing are inconclusive. Multiplex assays are especially suited for use in patients with complex sensitization patterns or symptoms, in small children with limited skin area, in elderly when skin test is less reliable, and when medications interfering with skin prick testing cannot be discontinued (5, 34).

We compared our results with other studies from the Middle-European region in the point of view the outcomes describing the sensitization patterns to molecular components (9, 35, 36). Panzner et al. investigated 1255 sensitized patients, with a mean age of 29 years, and with the following diagnoses: chronic rhinitis (73%), bronchial asthma (41%), atopic dermatitis (34%), urticaria or edema (19%), and/or anaphylaxis (11%) (35, 36). In our study, we investigated the group of patients suffering from atopic dermatitis, some of these patients suffer from bronchial asthma (55.8%) and from allergic rhinitis (76.0%). Our

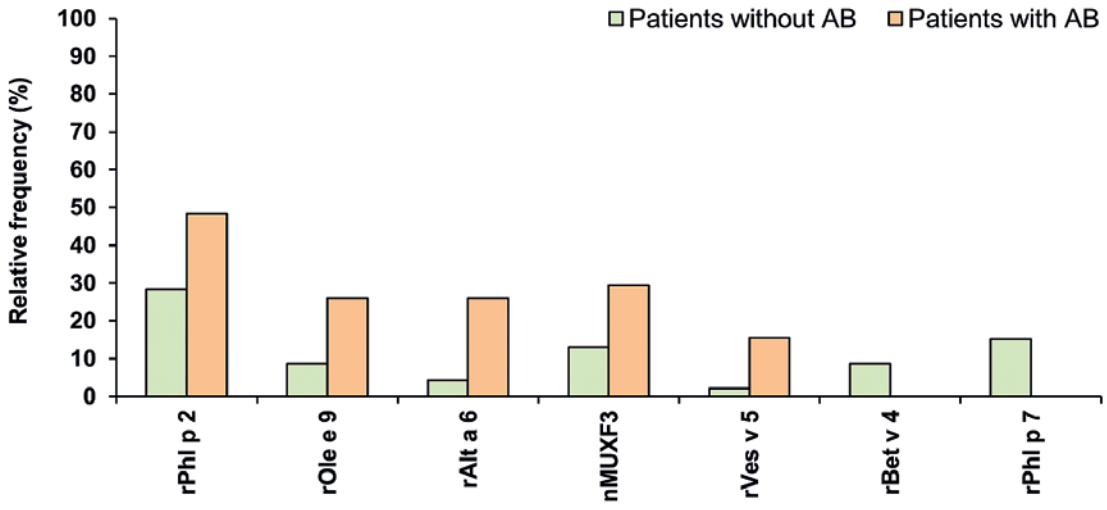

Fig. 3 related to Tab. 4: Sensitization to molecular components according to positivity (relative frequency) in subgroup of patients suffering from bronchial asthma – statistically significant difference (p-value < 0.05).

Tab. 5 The list of molecular components according to positivity (relative frequency) in subgroup of patients suffering from allergic rhinitis – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	*No.* of patients without AR (%) (25 patients = 100 %)	*No.* of patients with AR (%) (79 patients = 100 %)	p-value
Birch	**rBet v 1**	9 (36.0 %)	49 (62.0 %)	0.022
Hazel pollen	**rCor a 1.0101**	5 (20.0 %)	41 (51.9 %)	0.005
Apple	**rMal d 1**	6 (24.0 %)	42 (53.2 %)	0.011
Peach	**rPru p 1**	6 (24.0 %)	41 (51.9 %)	0.015
Celery	**rApi g 1**	1 (4.0 %)	21 (26.6 %)	0.016
Kiwifruit	**nAct d 1**	0 (0.0 %)	11 (13.9 %)	0.048
Bermuda grass	**nCyn d 1**	7 (28.0 %)	45 (57.0 %)	0.012
Timothy grass	**rPhl p 5**	5 (20.0 %)	38 (48.1 %)	0.013
Timothy grass	*rPhl p 6*	6 (24.0 %)	37 (46.8 %)	0.043
House dust mite	**rDer f 2**	7 (28.0 %)	40 (50.6 %)	0.048
Dog	**rCan f 1**	3 (12.0 %)	37 (46.8 %)	0.002
Cat	**rFel d 4**	2 (8.0 %)	28 (35.4 %)	0.008
Horse	**rEqu c 1**	3 (12.0 %)	26 (32.9 %)	0.042
Mouse	**nMus m 1**	1 (4.0 %)	20 (25.3 %)	0.021

AR – allergic rhinitis; major allergens are highlighted in bold (e.g. **rBet v 1**), minor allergens are highlighted in italics (e.g. *rPhl p 6*), and cross-reactive components are illustrate in grey box (e.g. **rMal d 1**)

results are in agreement with the Panzner's hypothesis (9) that grasses (rPhl p 1) and *Betulaceae* (rBet v 1) components comprised the vast majority of pollen sensitizations in the condition of Middle-European region. On the other hand, the sensitization to animal allergen molecules was higher in our study (to rFel d 1 in 42.0%, to rCan f 1 in 39.0%, to rFel d 4 in 29.0%, to rEqu c 1 in 27%, to rCan f 5 in 26%); the sensitization to mite molecular allergens was in our study higher also (to rDer p 2 in 46.0%, to rDer f 2 in 45%, to nDer p 1 in 36.0%, to nDer f 1 in 34.0%). In the Panzner's study, the sensitization rate to animal allergen molecules was confirmed to rFel d 1 in 31.8%, to rCan f 1 in 13.9%, to rCan f 5 in 16.4%, to rEqu c 1 in 6.2%, to rFel d 4 in 5.3% (36). The sensitization to at least one mite-specific molecule (nDer p 1, rDer p 2, nDer f 1, rDer f 2) was observed in 32.7% of patients in Panzner's study (35). The explanation of higher sensitization rate to animal and mite molecular allergens in our group of patients can be in the fact, that we included patients suffering from atopic dermatitis; in the Panzner's study, atopic dermatitis patients represent only 34% of patients. Our results may demonstrate the significance of disturbed epidermal barrier, resulting in increased transepidermal water loss and permeation of allergens, irritants, and microbes. It is evident that the direct contact of skin with allergens could trigger signals to initiate Th2 allergic response. Emerging data now suggest that epithelial cell-derived cytokines such as TSLP, IL-33, and IL-25 may drive the progression from atopic dermatitis to bronchial asthma and food allergy (1–3). In 2014, it was reported that IgE antibodies to Der p 11 are more common in

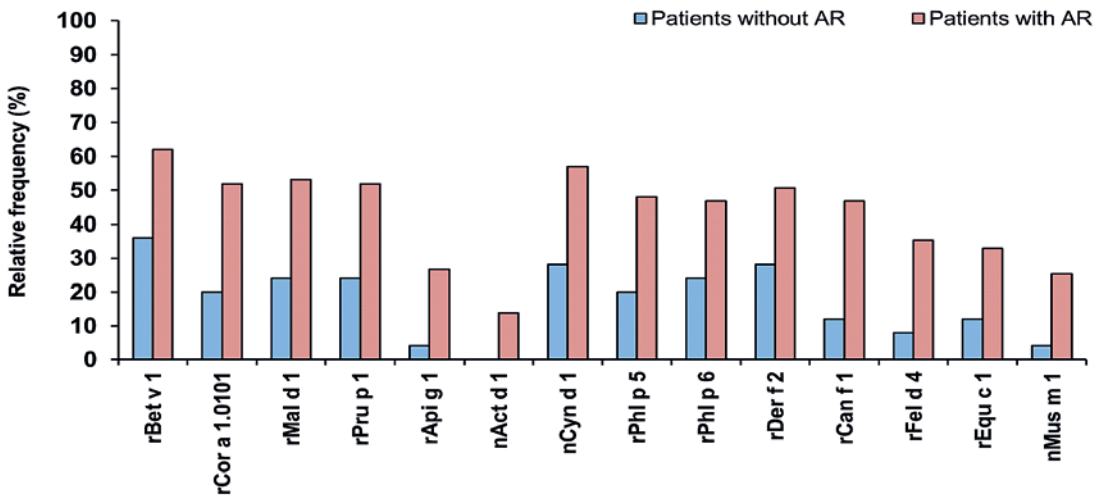

Fig. 4 related to Tab. 5: Sensitization to molecular components according to positivity (relative frequency) in subgroup of patients suffering from allergic rhinitis – statistically significant difference (p-value <0.05).

sera from patients with atopic dermatitis (37). Thus, sensitization to this allergen may reflect the fact that the eczematous skin allows easy penetration of allergens even with molecular weight as high as 100,000. In ISAC testing, the molecular component Der p 11 is not present, so we cannot compare it with our results. Although the house dust mite allergens are present in the mite bodies, the main allergenic sources are the mite faeces which, with a diameter higher than 10 μm (37), can be easily inhaled into the airways and consequently be entered deep into the lungs (37).

Our preliminary results regarding the analysis of sensitization to molecular components in atopic dermatitis patients were already published or they are in press (38, 39, 40, 41). We analysed the data to find the molecular components with the highest underlying probability of sensitization in patients suffering from atopic dermatitis and in subgroups of patients with allergic rhinitis and bronchial asthma (38). According to our results, the order of molecular components in mild form of AD is not statistically significant, but a set of molecular components with the highest underlying probability in moderate and severe form of AD and in a subgroup of patients suffering from allergic rhinitis was recorded (38). According to the statistical method with cluster analysis, we found 10 clusters with different numbers of molecular components (39). Fundamental position have the components rPhl p 1 (timothy), rBet v 1 (birch), rAlt a 1 (*Alternaria*) followed by molecular components of NPC2 family, cysteine protease, tropomyosin, uteroglobin, lipocalin and PR-10 protein. Our results correspond to the association of molecular components into protein families according to their biochemical structure (39). The preliminary data regarding the sensitization to molecular components in 81 atopic dermatitis patients were processed in other publications (40, 41).

There are various allergens that can trigger an eczema flare up. An allergen-specific IgE-mediated response to a wide spectrum of food and inhalant allergens, especially house dust mite, pollen and plant-derived food allergens, has been described in adult AD patients (8). The aim of our study was to identify some differences in the occurrence of the sensitization to the molecular components in the group of 104 atopic dermatitis patients in relation to severity of AD and to the occurrence of bronchial asthma and allergic rhinitis.

According to our results, rPhl p 1 is a leading molecular component in patients suffering from severe form of AD as well as in subgroups of patients with AR and AB. However, the occurrence of rPhl p 1 was significantly more frequent only in patients with severe form of AD. The IgE response usually evolves from monosensitization to polysensitization, this phenomenon has been described as "molecular spreading". rPhl p 1 (beta-expansin) is a presumable the "initiator" of the sensitization process in most patients. It is the major grass-specific allergen belongs to grass group 1. In addition, it is an essential diagnostic marker for allergic patients to establish "true sensitization" to grass pollen (timothy). In a few cases the grass pollen allergy might be evoke by isolated IgE sensitization to another major grass-specific allergen (e.g. rPhl p 5), but it is rather unlikely (34). nPhl p 4 is a minor grass-specific allergen, a highly glycosylated protein, that can bind to IgE specific for cross-reactive carbohydrate determinants (CCDs) (5). High sensitization rate to nPhl p 4 was observed in our study in subgroup of patients with AR, but there was no evidence of significant difference in comparison to patients without AR. The major allergen of bermuda grass pollen is a nCyn d 1 from the beta-expansin family that is commonly found in subtropical regions but is not presented in our region (rarely in south Moravia) (9). Possible cross-reactivity with beta-expansins from other grasses, especially with the major allergen of timothy could be the explanation of higher occurrence of nCyn d 1 in subgroup of AR patients in our study. Sensitization to other components from the same pollen source usually come before the sensitization to panallergens (e.g. polcalcins and profilins) which are typically recognized at the late stage of molecular spreading (29, 42). Specific IgE to rPhl p 7 determine a relatively distinct category of grass pollen allergic patients, who may suffer from more severe symptoms, with a higher prevalence of bronchial asthma, and a higher frequency of other allergic comorbidities (34). In contrast to these findings, our subgroup of patients with bronchial asthma showed no positive results of sIgE against polcalcins rPhl p 7 and rBet v 4 ($p < 0.05$).

Surprisingly, not negligible sensitization rate to component of olive rOle e 9 was observed more frequently in patients with severe form of AD and in subgroup of patients with AB; rOle e 9 (glucanase) is a major olive allergen which commonly cause sensitivity in geographical areas exposed to high levels of olive pollen (43). Moreover, rOle e 9 shares some common epitopes with glucanases from birch and ash pollens, tomato, potato, pepper, banana, and latex (44) that might be the explanation of higher sensitization rate to this component.

Our results pointed out that the sensitization to major components of mites in severe form of AD might be associated with the sensitization to major components of animals (rCan f 1, rCan f 5, rFel d 1, rFel d 4, rEqu c 1, nMus m 1) and minor components of moulds (rAlt a 6, rAsp f 6). Animals are the second most important source of indoor allergens after house dust mites (45). They are considered as risk factors for the development of allergic rhinitis and asthma (46). Numbers of dog, cat, and horse allergens have been described. Vast majority belongs to the protein families of uteroglobin, lipocalin and kallikrein. We observed the high sensitization rates to lipocalins and uteroglobins. Lipocalins represent the most important protein family, which are synthesized in salivary glands. Most of them are major animal allergens (rCan f 1, rFel d 4, rEqu c 1, nMus m 1). Can f 5, considered as a major dog allergen, is a prostatic kallikrein (arginine esterase) that is found only in male dogs (34, 47). Fel d 1, a major cat allergen, is a uteroglobin expressed in salivary glands and skin. The severity of induced symptoms varies widely and cat and dog allergy could be the principal risk factor of both rhinitis and asthma, associated with higher severity, which can develop into a life-threatening condition (45, 47). It is in an agreement with our results. House dust mites (HDM) belong to the most potent indoor allergen sources that are associated with allergic manifestations in the respiratory tract and the skin (37). The effect of mites on the human organism is complex because of mites can carry microbial and fungal

antigens, respectively pathogen-associated molecular patterns (PAMPs), thus initiating mechanisms of innate immunity. The largest number of HDM molecules is known in the two most important species *Dermatophagoides* (*D.*) *farinae* and *D. pteronyssinus* (34). Their molecular components can be divided into groups according to protein families. Group 1 (cysteine proteases) includes the major molecular components of nDer f 1 and nDer p 1, which show 85% homology (35). Group 2 (NPC2 family) comprise the major components rDer f 2 and rDer p 2, which show up to 90% homology within the group (35). These allergens are assumed to be the specific components for mite allergy. The presence of sIgE to major molecular components nDer p 1, rDer p 2 (34) and Der p 23 (48), that are present in fecal particles of mites, has strong association with asthma. However, this is not in the concordance with our results. We recorded that the sensitization to group 2 allergens was significantly higher only in patients with severe form of AD (rDer f 2 and rDer p 2) and in subgroup of patients suffering from allergic rhinitis (rDer f 2). Recently identified Der p 11 is present predominantly in the muscle of HDM bodies that belong to the family of proteins known as paramyosins. Der p 11 seems to be a useful serological marker for HDM-allergic patients suffering from atopic dermatitis (37). Unfortunately, molecular components Der p 23 and Der p 11 are not included in the ISAC test, thus we cannot compare them with our results. A strong immunogenic potential of mite components may play a crucial role in the atopic march (49). Moreover, an impaired epidermal barrier is considered as the most important factor that elicit atopic march (50). The prevalence of mould sensitization displays wide geographical variability (34). Considering the sensitization to minor components rAsp f 6 (*Aspergillus fumigatus*, Mn superoxide dismutase) and rAlt a 6 (*Alternaria alternata*, enolase) were recorded with significantly higher occurrence in patients suffering from severe form of AD (rAsp f 6, rAlt a 6) and in subgroup of patients with bronchial asthma (rAlt a 6). Sensitization to *Alternaria* (*A.*) *alternata* is a risk factor to develop asthma (51). Furthermore, bronchial asthma is characterized by more persistent symptoms and enhanced disease severity. *A. alternata* is a widespread saprophyte that is usually found in outdoor, however, it can also occur in indoor environments. Moreover, sensitization to *A. alternata* seems to be a triggering factor in the development of poly-sensitization (52). Nevertheless, the clinical relevance of high level of specific IgE to *Alternaria* in patients with AD remains unclear (53). *Aspergillus* (*A.*) *fumigatus* is a mould permanently present in the indoor and outdoor environment (34). Aspergillus allergy is rare in atopic individuals without asthma or cystic fibrosis (54). Interestingly, the phylogenetically highly conserved allergens Asp f 6, Asp f 8, Asp f 11, Asp f 27, Asp f 28 and Asp f 29 show a high degree of cross-reactivity with other mould proteins belonging to the same families. The clinical relevance of these reactions remains elusive (34).

Bet v 1 homologous allergens (PR-10 like proteins) shows a highly cross-reactivity pattern. Birch (rBet v 1), followed by alder (rAln g 1) and hazel (rCor a 1.0101) constitute the most potent cause of tree pollen allergy (55). According to our result, there is no relation between the subgroup of patients with AB and the sensitization to *Betulaceae*-specific components. The significantly higher occurrence of sensitization to PR-10 proteins was recorded to rApi g 1 (celery) in patients suffering from severe form of AD and to major components of tree-specific components, such as rBet v 1 (birch), rCor a 1.0101 (hazel), and pollen-food derived proteins like rMal d 1 (apple), rPru p 1 (peach), and rApi g 1 (celery) in subgroup of patients suffering from AR. These pollen-food derived PR-10 proteins mainly cause local manifestations of allergic reactions and may induce a variety of "pollen-food" syndromes (8). Results of sensitization profile in the subgroup of patients with AR suggest that AR patients are mostly sensitized to inhalant allergens (pollen or pollen-food derived PR-10) via the respiratory tract or digestive system. Röckmann et al. (8) demonstrated that sensitization to food-derived PR-10 allergens occurred most frequently in AD patients but there was no association between their presence and severity of AD. They recorded higher sensitization rate to rAra h 1 (peanut) and nBos d lactofferin (cow's milk) in patients with severe form of AD. We recorded in the higher frequency the presence of specific IgE to major food allergens of wheat (nTri a aA_TI), egg white (nGal d 2), walnut (nJug r 2) and peanut (rAra h 1) but only in patients with severe form of AD. The outcome of our analysis could be influenced by the fact that some allergens showed zero frequencies of positive values which were particularly evident when we have compared mild, moderate and severe form of AD. The molecular component nTri a aA_TI is a part of the ISAC assay and it is a trypsin/α-amylase inhibitor of wheat grains. Its designation is not based on the official WHO/IUIS database (www.allergen.org), but it names from Phadia. Peanut allergens are the most common trigger of food-induced anaphylaxis (34). rAra h 1 (7S globulin) is a thermostable seed storage protein whose allergenicity can be increased by roasting (34). nGal d 2 (ovalbumin), a major allergen, is the most abundant egg white protein. It is less heat-stable than ovomucoid (nGal d 1). IgE responses to Gal d 2 indicate a risk for clinically relevant reaction to raw or slightly heated egg (34, 56). nJug r 2, a highly glycosylated protein, has been identified as an important allergen in common walnut. Native vicilin-like protein nJug r 2 (7S globulin), can bind to IgE specific for cross-reactive carbohydrate determinants (CCDs), and can also be raised in patients sensitized to CCDs (57). For this reason, the real clinical significance of a positive nJug r 2 result must be carefully evaluated in the context of the results of other components and clinical findings (5).

Food allergy to shellfish (crustaceans and molluscs) may cause cross-sensitization and clinical reactivity to house dust mites, insects and arachnids (34). Numbers of crustacean allergens have been described. Tropomyosin is a major shrimp allergen. Several others allergenic components have been identified (arginine kinase, myosin light chain and sarcoplasmic calcium binding protein) (34). Our result showed the higher occurrence of a minor shrimp allergen nPen m 2 (arginine kinase) in patients suffering from severe form of AD. Similar to tropomyosin, arginine kinase is highly abundant in invertebrate muscle, and up to now it has been described in various shellfish and other invertebrates such as mites, cockroaches, crab, shrimp,

and crayfish (58, 59). Unlike tropomyosins, they show termolabile properties (60).

Component-resolved diagnostics seems to be a promising tool for the diagnosis of food allergy, offering the potential to determine specific phenotypes and estimate the risk of immune response to a given allergen. Nevertheless, the diagnostic accuracy of these laboratory tests varies across studies. Therefore, their clinical utility remains unclear (61). The assessment of diagnostic accuracy (sensitivity, specificity) for certain food allergen components (ISAC test) will be the topic for our future research.

CONCLUSIONS

Sensitization to following molecular components of grasses (rPhl p 1), trees (rOle e 9, rBet v 2), house dust mites (rDer f 2, rDer p 2), animals (rFel d 1, rFel d 4, rCan f 1, rCan f 5, rEqu c 1, nMus m 1), moulds (rAlt a 6, rAsp f 6), and foods (nTri a aA_TI, nGal d 2, rAra h 1, nJug r 2, nPen m 2, rApi g 1) was significantly more frequent in patients with severe form of AD. In this regard, we recommend enrolling the assessment of the presence of specific IgE against these components into the clinical procedures and treatment of allergy patients with the special respect to the risk of development of severe reactions.

Typically, in subgroup of patients suffering from allergic rhinitis the significantly higher sensitization to molecular components, such as tree pollen (rBet v 1, rCor a 1.0101), grass pollen (rPhl p 5, rPhl p 6), house dust mites (rDer f 2), animals (rCan f 1, rFel d 4, rEqu c 1, nMus m 1), and foods (rMal d 1, rPru p 1, rApi g 1, nAct d 1) is recorded in our study. In patients with bronchial asthma the significantly higher sensitization to molecular components of grasses (rPhl p 2), trees (rOle e 9) and *Alternaria* (rAlt a 6) was recorded. These molecular components may play the important role in the atopic march in an individual patient.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Czarnowicki T, Krueger JG, Guttman-Yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations with implications for the atopic march. J Allergy Clin Immunol 2017; 139(6): 1723–34.
2. Paller AS, Spergel JM, Mina-Osorio P, Irvine AD. The atopic march and atopic multimorbidity: Many trajectories, many pathways. J Allergy Clin Immunol 2019; 143(1): 46–55.
3. Zhu TH, Zhu TR, Tran KA, Sivamani RK, Shi VY. Epithelial barrier dysfunctions in atopic dermatitis: a skin–gut–lung model linking microbiome alteration and immune dysregulation. Br J Dermatol 2018; 179(3): 570–81.
4. Krejsek J, Andrýs C, Krčmová I. Imunologie člověka. In Hradec Králové: Garamon; 2016, p. 213–22.
5. Ansotegui IJ, Melioli G, Walter G, et al. Open Access A WAO–ARIA–GA2LEN consensus document on molecular-based allergy diagnosis (PAMD@): Update 2020. World Allergy Organ J 2020; 13(2): 1–46.
6. Zheng T, Yu J, Oh MH, Zhu Z. The atopic march: Progression from atopic dermatitis to allergic rhinitis and asthma. Allergy, Asthma Immunol Res 2011; 3(2): 67–73.
7. He Y, Liu X, Huang Y, Zou Z, et al. Reduction of the number of major representative allergens: From clinical testing to 3-dimensional structures. Mediators Inflamm 2014; 2014: 1–11.
8. Röckmann H, Van Geel MJ, Knulst AC, Huiskes J, Bruijnzeel-Koomen CAFM, De Bruin-Weller MS. Food allergen sensitization pattern in adults in relation to severity of atopic dermatitis. Clin Transl Allergy 2014; 4(1): 1–9.
9. Panzner P, Vachová M, Vítovcová P, Brodská P, Vlas T. A comprehensive analysis of middle-european molecular sensitization profiles to pollen allergens. Int Arch Allergy Immunol 2014; 164(1): 74–82.
10. Patelis A, Borres MP, Kober A, Berthold M. Multiplex component-based allergen microarray in recent clinical studies. Clin Exp Allergy 2016; 46(8): 1022–32.
11. Mothes-Luksch N, Jordakieva G, Hinterhölzl L, et al. Allergy diagnosis from symptoms to molecules, or from molecules to symptoms: A comparative clinical study. World Allergy Organ J 2018; 11(1): 22.
12. Passalacqua G, Melioli G, Bonifazi F, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. Allergy Eur J Allergy Clin Immunol 2013; 68(8): 1029–33.
13. Foong RX, Roberts G, Fox AT, Toit G. Pilot study: Assessing the clinical diagnosis of allergy in atopic children using a microarray assay in addition to skin prick testing and serum specific IgE. Clin Mol Allergy 2016; 14(1): 1–7.
14. Choi J, Roh J, Lee J. Clinical Availability of Component-Resolved Diagnosis Using Microarray Technology in Atopic Dermatitis. Ann Dermatol 2014; 26(4): 437–46.
15. van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: Pros and cons in allergology. J Allergy Clin Immunol 2017; 140(4): 974–7.
16. Melioli G, Bonifazi F, Bonini S, et al. The ImmunoCAP ISAC molecular allergology approach in adult multi-sensitized Italian patients with respiratory symptoms. Clin Biochem 2011; 44(12): 1005–11.
17. Wojciechowska M, Żbikowska-Gotz M, Marek-Józefowicz L, Przybyszewski M, Gocki J, Bartuzi Z. Allergic phenotypes in adult patients with atopic dermatitis, determined with the ISAC test (ImmunoCAP ISAC). Postep Dermatologii i Alergol 2018; 35(4): 351–9.
18. Ott H, Fölster-Holst R, Merk HF, Baron JM. Allergen microarrays: A novel tool for high-resolution IgE profiling in adults with atopic dermatitis. Eur J Dermatology 2010; 20(1): 54–61.
19. Hanifin JM, Rajla G. Diagnostic features of AD. Vol. 92, Acta Dermatovener (Stockholm), 1980, p. 44–7.
20. Severity scoring of atopic dermatitis: the SCORAD Index (Consensus report of the European Task Force on Atopic Dermatitis). Dermatology 1993; 186: 23–31.
21. Oranje AP, Glazenburg EJ, Wolkerstorfer A, De Waard-Van Der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: The SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 2007; 157(4): 645–8.
22. Martínez-Aranguren R, Lizaso MT, Goikoetxea MJ, et al. Is the determination of specific IgE against components using ISAC 112 a reproducible technique? PLoS One 2014; 9(2): 1–7.
23. Jakob T, Forstenlechner P, Matricardi P, Kleine-Tebbe J. Molecular allergy diagnostics using multiplex assays : methodological and practical considerations for use in research and clinical routine. Allergo J Int 2015; 24: 320–32.
24. Horimukai K, Morita K, Narita M, et al. Transepidermal water loss measurement during infancy can predict the subsequent development of atopic dermatitis regardless of filaggrin mutations. Allergol Int 2016; 65(1): 103–8.
25. Dodig S, Čepelak I. The potential of component-resolved diagnosis in laboratory diagnostics of allergy. Biochem Medica 2018; 28(2): 1–9.
26. Boyce JA. Guidelines for the diagnosis and management of food allergy in the United States: Report of the NIAID-sponsored expert panel. J Allergy Clin Immunol 2010; 126(6): 301–402.
27. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI Food Allergy and Anaphylaxis Guidelines: Diagnosis and management of food allergy. Allergy Eur J Allergy Clin Immunol 2014; 69(8): 1008–25.
28. Prosperi MCF, Belgrave D, Buchan I, Simpson A, Custovic A. Challenges in interpreting allergen microarrays in relation to clinical symptoms: A machine learning approach. Pediatr Allergy Immunol 2014; 25(1): 71–9.

29. Hatzler L, Panetta V, Lau S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin Immunol 2012; 130(4): 894–901.
30. Williams P, Önell A, Baldracchini F, Hui V, Jolles S, El-Shanawany T. Evaluation of a novel automated allergy microarray platform compared with three other allergy test methods. Clin Exp Immunol 2016; 184(1): 1–10.
31. Huss-Marp J, Gutermuth J, Schäffner I, et al. Comparison of molecular and extract-based allergy diagnostics with multiplex and singleplex analysis. Allergo J Int 2015; 24(2): 46–53.
32. Ahlgrim C, Gutermuth J, Önell A, et al. Comparison of Molecular Multiplex and Singleplex Analysis of IgE to Grass Pollen Allergens in Untreated German Grass Pollen – Allergic Patients. J Investig Allergol Clin Immunol 2015; 25(3): 190–5.
33. Jung JH, Kang G, Kim TS. Comparison of Component-Resolved Diagnosis by Using Allergen Microarray With the Conventional Tests in Allergic Rhinitis Patients : The First Using in Korea. Clin Exp Otorhinolaryngol 2015; 8(4): 385–9.
34. Matricardi PM, Hoffmann HJ, Valenta R, et al. EAACI Molecular Allergology User's Guide. Pediatr Allergy Immunol 2016; 27(23): 1–250.
35. Panzner P, Vachová M, Vlas T, Vítovcová P, Brodská P, Malý M. Cross-sectional study on sensitization to mite and cockroach allergen components in allergy patients in the Central European region. Clin Transl Allergy 2018; 8(19): 1–9.
36. Vachová M, Panzner P, Vlas T, Vítovcová P. Analysis of Sensitization Profiles in Central European Allergy Patients Focused on Animal Allergen Molecules. Int Arch Allergy Immunol 2020; 181(4): 278–84.
37. Banerjee S, Resch Y, Chen KW, et al. Der p 11 is a Major Allergen for House Dust Mite-Allergic Patients Suffering from Atopic Dermatitis. J Invest Dermatol 2015; 135(1): 102–9.
38. Čelakovská J, Bukač J, Vaňková R, Krčmová I, Krejsek J, Andrýs C. ISAC multiplex testing – results of examination in 100 patients suffering from atopic dermatitis. Food Agric Immunol 2020; 31(1): 1014–35.
39. Čelakovská J, Bukač J, Vaňková R, et al. Cluster analysis of molecular components in 100 patients suffering from atopic dermatitis according to the ISAC Multiplex testing. Food Agric Immunol 2020; 31(1): 810–31.
40. Čelakovská J, Bukač J, Vaňková R, Krčmová I, Krejsek J, Andrýs C. Sensitisation to molecular components in patients with atopic dermatitis, relation to asthma bronchiale and allergic rhinitis. Food Agric Immunol 2020; 31(1): 600–29.
41. Vaňková R, Čelakovská J, Bukač J, Krčmová I, Krejsek J, Andrýs C. Využití multiplexového systému ImmunoCAP ISAC k určení senzibilizace na molekulární komponenty související se závažností atopické dermatitidy. Čes-slov Derm, in print.
42. Nicoló S, Braun T, Eder K, Berghaus A, Gröger M. Clinical Relevance of IgE to Profilin and/or Polcalcin in Pollen-Sensitized Patients. Int Arch Allergy Immunol 2016; 169: 101–7.
43. Rodríguez R, Villalba M, Batanero E, et al. Allergenic diversity of the olive pollen. Allergy Eur J Allergy Clin Immunol Suppl 2002; 57(71): 6–16.
44. Palomares O, Villalba M, Quiralte J, Polo F, Rodrı R. 1,3-β-glucanases as candidates in latex–pollen–vegetable food cross-reactivity. Clin Exp Allergy 2005; 35: 345–51.
45. Bonnet B, Messaoudi K, Jacomet F, et al. An update on molecular cat allergens: Fel d 1 and what else? Chapter 1: Fel d 1, the major cat allergen. Allergy, Asthma Clin Immunol 2018; 14(14): 1–9.
46. Konradsen JR, Fujisawa T, Hage M Van, Hedlin G. Current perspectives Allergy to furry animals: New insights, diagnostic approaches, and challenges. J Allergy Clin Immunol 2015; 135(3): 616–25.
47. Obando SU, Domínguez JS. Clinical impact of molecular diagnosis in dog allergy. Clin Transl Allergy 2014; 4(Suppl 2): P52.
48. Celi G, Brusca I, Scala E, et al. House dust mite allergy in Italy—Diagnostic and clinical relevance of Der p 23 (and of minor allergens): A real-life, multicenter study. Allergy Eur J Allergy Clin Immunol 2019; 74(9): 1787–9.
49. Posa D, Perna S, Resch Y, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. J Allergy Clin Immunol 2017; 139(2): 541–9.
50. Weidinger S, Illig T, Baurecht H, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006; 118(1): 214–9.
51. Feo Brito F, Alonso AM, Carnés J, et al. Correlation between Alt a 1 levels and clinical symptoms in Alternaria alternata- monosensitized patients. J Investig Allergol Clin Immunol 2012; 22(3): 154–9.
52. Gabriel MF, Postigo I, Tomaz CT, Martínez J. Alternaria alternata allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environ Int 2016; 89–90: 71–80.
53. Vailes LD, Perzanowski MS, Wheatley LM, Platts-Mills TAE, Chapman MD. IgE and IgG antibody responses to recombinant Alt a 1 as a marker of sensitization to Alternaria in asthma and atopic dermatitis. Clin Exp Allergy 2001; 31(12): 1891–5.
54. Crameri R, Blaser K. Allergy and immunity to fungal infections and colonization. Eur Respir J 2002; 19(1): 151–7.
55. Scala E, Cecchi L, Guerra C. Molecular Recognition Profiles and Clinical Patterns of PR-10 Sensitization in a Birch-Free Mediterranean Area. Int Arch Allergy Immunol 2017; 173: 138–46.
56. Petrosino MI, Scaparrotta A, Marcovecchio ML, et al. Usefulness of molecular diagnosis in egg allergic children. Arch Med Sci 2018; 14(1): 132–7.
57. Villalta D, Conte M, Asero R, Re M Da, Stella S. Isolated IgE reactivity to native walnut vicilin-like protein (nJug r 2) on ISAC™ microarray is due to cross-reactive carbohydrate epitopes. Clin Chem Lab Med 2013; 51(10): 1991–5.
58. Yu C-J, Lin Y-F, Chiang B-L, Chow L-P. Proteomics and Immunological Analysis of a Novel Shrimp Allergen, Pen m 2. J Immunol 2003; 170(1): 445–53.
59. Faber MA, Pascal M, Kharbouchi O El, et al. Shellfish allergens: tropomyosin and beyond. Allergy 2017; 72: 842–8.
60. Lopata AL, Kleine-Tebbe J, Kamath SD. Allergens and molecular diagnostics of shellfish allergy. Allergo J Int 2016; 25(7): 210–8.
61. Kim JF, Mccleary N, Nwaru BI, Stoddart A, Sheikh A. Diagnostic accuracy, risk assessment, and cost-effectiveness of component-resolved diagnostics for food allergy: A systematic review. Allergy Eur J Allergy Clin Immunol 2018; 73: 1609–21.