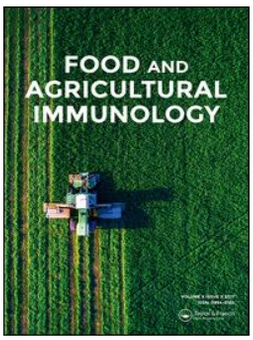

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Sensitisation to molecular allergens of Alternaria alternata, Cladosporium herbarum, Aspergillus fumigatus in atopic dermatitis patients

Jarmila Čelakovská[a], Josef Bukač[b], Radka Vaňková[c], Irena Krcmova[c], Jan Krejsek[c] and Ctirad Andrýs[c]

[a]Department of Dermatology and Venereology, Faculty Hospital and Medical Faculty of Charles University, Hradec Králové, Czech Republic; [b]Department of Medical Biophysic, Medical Faculty of Charles University, Hradec Králové, Czech Republic; [c]Department of Clinical Immunology and Allergy, Faculty Hospital and Medical Faculty of Charles University, Hradec Králové, Czech Republic

ABSTRACT

The aim of this study is the evaluation of the sensitisation to molecular allergens of Alternaria alternata, Aspergillus fumigatus and Cladosporium herbarum in atopic dermatitis patients. The complete dermatological and allergological examination including the examination of the sensitisation to molecular allergens with Multiplex ISAC testing was performed in all included patients. The statistical evaluation of the relation between the sensitisation to Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6 and other molecular food and inhalant allergens was performed. The sensitisation to molecular fungal allergen was recorded altogether in 58% patients; it is in significant relation to the sensitisation to some food and inhallant molecular allergens, such as Peanut, Walnut, Hazelnut, Atlantic Cod, Black Tiger Shrimp, Apple, Kiwi, Peach, Celery, Timothy, London Plane tree, Cedar pollen allergen, Mugwort, Cat, and Horse.

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KEYWORDS
Atopic dermatitis; Alternaria alternata; Aspergillus fumigatus; Cladosporium herbarum; molecular allergens; multiplex ISAC testing

Introduction

Atopic dermatitis constitutes together with allergic rhinitis and asthma the triad of atopic diseases. The pathogenesis of atopic dermatitis involves interactions among multiple factors including susceptibility genes, environmental factors (food and inhallant allergens), skin barrier defects, and immunologic factors (Boguniewicz & Leung, 2010; Leung & Bieber, 2003). Exposure and sensitisation to fungal allergens can promote the development and worsening of allergic diseases. Although numerous species of fungi have been associated with allergic diseases in the literature, the significance of fungi from the genera Alternaria, Cladosporium, Penicillium, Aspergillus, and Malassezia has been well documented. Despite its importance in the management of allergic diseases, precise recognition of species-specific IgE sensitisation to fungal allergens is often challenging because the

CONTACT Jarmila Čelakovská jarmila.celakovska@seznam.cz

majority of fungal extracts exhibit broad cross-reactivity with taxonomically unrelated fungi. Recent progress in gene technology has contributed to the identification of specific and cross-reactive allergen components from different fungal sources. However, data demonstrating the clinical relevance of IgE reactivity to these allergen components are still insufficient (Fukutomi & Taniguchi, 2015). The initial laboratory approach in the diagnosis of allergies (such as atopic dermatitis, rhinitis and wheezing disorders) is to detect the type of allergic reaction, i.e. whether the patient's allergy is mediated by immunoglobulin E (IgE) or not. Progress in laboratory diagnostics of IgE-mediated allergy is the use of component-resolved diagnosis (CRD) or molecular diagnosis of allergies. The CRD approach has been developed when highly purified or recombinant allergen molecules have become available. These molecules are the allergenic proteins toward which the specific and clinically relevant IgE immune response is directed. The introduction of allergen molecules has had a major effect on analytic specificity and allergy diagnosis. They are used in both singleplex ImmunoCAP and multiplex ImmunoCAP ISAC assays. The major advantage of ISAC is the comprehensive IgE pattern obtained with a minute amount of serum (van Hage, Hamsten, & Valenta, 2017). ImmunoCAP ISAC (Thermo Fisher), based on 112 different molecular components (both extracted and recombinant), is the most studied and most frequently used molecular diagnostic tool based on a microarray (Melioli et al., 2011). The goal of CRD is to distinguish the true allergens from the cross-reactive allergen molecules. Determination of sIgE against allergenic components may significantly improve current diagnostics of allergy (Dodig & Čepelak, 2018 Jun 15). The main allergenic sources are foods, fungi, trees, weeds, grasses, mites, and finally animals; with the largest number of allergenic proteins being found in foods and the smallest in animals (He et al., 2014). Currently allergens could be defined as proteins, glycoproteins, lipoproteins, or protein-conjugated haptens, which have unique molecular and structural properties. It has been largely demonstrated that fungi are potent sources of allergenic molecules covering a vast variety of molecular structures including enzymes, toxins, cell wall components and phylogenetically highly conserved cross-reactive proteins (Simon-Nobbe, Denk, Poll, Rid, & Breitenbach, 2008). The official WHO/IUIS database (www.allergen.org) currently lists 77 mould allergens from a variety of protein families. To date, only eight recombinant single allergens from three mould species are available for molecular allergy diagnosis of mould sensitisation. These include rAlt a 1, the major allergen in *Alternaria alternata*-sensitized individuals, and enolase rAlt a 6 with it potential cross-reactivity to mould, food and natural latex allergens. Molecular allergens rAsp f 1, 2, 3, 4 and 6 from *Aspergillus fumigatus* are available for diagnostic purposes. The dehydrogenase r Cla h 8 is considered a major allergen of *Cladosporium herbarum* with possible cross-reactivity to other dehydrogenase allergens (Kespohl & Raulf, 2014).

The aim of this study is to show in group of patients suffering from atopic dermatitis the sensitisation to molecular allergens of Alternaria alternata (Alt a 1, Alt a 6), Cladosporium herbarum (Cla h 8), Aspergillus fumigatus (Asp f 1, Asp f 3, Asp f 6) and to evaluate the relation between the sensitisation to these molecular fungal allergens and other molecular food and inhalant allergens according to CRD examination.

In our previous publications, we demonstrated our results regarding the sensitisation to fungi in atopic dermatitis patients with the examination of skin prick test and specific IgE (Čelakovská, Bukač, Ettler, Vaneckova, Krcmova, et al., 2018; Čelakovská, Bukač, Ettler, Vaneckova, Ettlerova, et al., 2018).

Patients and methods

In the period 2018–19, 60 patients suffering from atopic dermatitis at the age of 14 years and older were examined. All these patients were examined in the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech republic. The diagnosis of atopic dermatitis was made with the Hanifin-Rajka criteria. Exclusion criteria were long term therapy with cyklosporin or systemic corticoids, pregnancy, breastfeeding. Patients with atopic dermatitis 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 commitee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech republic.

Examination of sIgE with to molecular allergens

The serum level of the sIgE was measured by the CRD microarray-based sIgE detection assay ImmunoCAP ISAC (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP ISAC is a solid-phase multiple immunoassay which enables determin 112 different components from 51 allergen sources (Jakob, Forstenlechner, Matricardi, & Kleine-Tebbe, 2015). The allergens are applied in triplicates to ensure the test reproducibility. The specific IgE values are presented in arbitrary units called ISAC Standardized Units (measuring range of 0.3–100 ISU-E). The list of the allergen components sorted by protein group is shown in Table 1. The level of specific IgE higher than 0.30 ISU-E was assessed as positive (Choi, Roh, & Lee, 2014). The statistical evaluation of the relation between the sensitisation to Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6 and different food and inhalant allergens according to the CRD examination was performed

Severity of atopic dermatitis

Severity of atopic dermatitis was scored in agreement with SCORAD (Scoring of atopic dermatitis) with the assessment of topography items (affected skin area), intensity criteria and subjective parameters. This examination was performed during one year every three month and the average SCORAD index was recorded. The severity of atopic dermatitis was evaluated with SCORAD as a mild form to 25 points, as moderate over 25–50 points, as a severe form over 50 points.

Statistical analysis

We analysed the data to determine whether the occurrence of sensitisation to Alt a 1, Alt a 6, Asp f 1, Asp f 3, or Asp f 6, Cla h 8 is associated with sensitisation to other molecular food and inhalant allergens. We used the statistical calculation with matrix. A matrix is a rectangular array of numbers arranged in rows and columns. Numbers that appear in the rows and columns of a matrix are called elements of the matrix. Also, we calculated contingency tables, such as Alt a 1 versus other allergents etc., and used the chi-squared test. The null hypothesis was the independence, the significance level was set to 1%, we used only 0 or 1 categories to avoid entries in the tables with low frequencies.

Table 1. The list of the allergen components sorted by protein group.

Allergen source	Allergen component	Protein group
Food components		
Egg white	nGal d 1	Ovomucoid
	nGal d 2	Ovalbumin
	nGal d 3	Conalbumin/ Ovotransferrin
Egg yolk/chicken meat	nGal d 5	Livetin/Serum albumin
Cow's milk	nBos d 4	Alpha-lactalbumin
	nBos d 5	Beta-lactoglobulin
	nBos d 8	Casein
	nBos d lactoferrin	Transferrin
Cod	rGad c 1	Parvalbumin
Shrimp	nPen m 2	Arginine kinase
	nPen m 4	Sarcoplasmic calcium binding protein
Cashew nut	rAna o 2	Storage protein, 11S globulin
Brazil nut	rBer e 1	Storage protein, 2S albumin
Hazelnut	nCor a 9	Storage protein, 11S globulin
Walnut	rJug r 1	Storage protein, 2S albumin
	nJug r 2	Storage protein, 7S globulin
Sesame seed	nSes i 1	Storage protein, 2S albumin
Peanut	rAra h 1	Storage protein, 7S globulin
	rAra h 2	Storage protein, 2S albumin
	rAra h 3	Storage protein, 11S globulin
	nAra h 6	Storage protein, 2S albumin
Soybean	nGly m 5	Storage protein, Beta-conglycinin
	nGly m 6	Storage protein, Glycinin
Buckwheat	nFag e 2	Storage protein, 2S albumin
Wheat	rTri a 14	Lipid transfer protein (nsLTP)
	rTri a 19.0101	Omega-5 gliadin
	nTri a aA_TI	Alpha-amylase/ Trypsin inhibitor
Kiwi	nAct d 1	Cysteine protease
	nAct d 5	Kiwellin
Aeroallergen components		
Grass pollen		
Bermuda grass	nCyn d 1	Grass group 1
Timothy grass	rPhl *p* 1	Grass group 1
	rPhl *p* 2	Grass group 2
	nPhl *p* 4	Berberine bridge enzyme
	rPhl *p* 5	Grass group 5
	rPhl *p* 6	Grass group 6
	rPhl *p* 11	Ole e 1-related protein
Tree pollen		
Birch	rBet v 1	PR-10 protein
Japanese cedar	nCry j 1	Pectate lyase
Cypress	nCup a 1	Pectate lyase
Olive pollen	rOle e 1	Common olive group 1
	rOle e 9	Beta-1,3-glucanase
Plane tree	rPla a 1	Putative ivertase inhibitor
	nPla a 2	Polygalacturonase
Weed pollen		
Ragweed	nAmb a 1	Pectate lyase
Mugwort	nArt v 1	Defensin
Goosefoot	rChe a 1	Ole e 1-related protein
Wall pellitory	rPar j 2	Lipid transfer protein (nsLTP)
Plantain	rPla l 1	Ole e 1-related protein
Saltwort	nSal k 1	Pectin methylestetrase
Animal		
Dog	rCan f 1	Lipocalin
	rCan f 2	Lipocalin
	rCan f 5	Arginine esterase
Horse	rEqu c 1	Lipocalin
Cat	rFel d 1	Uteroglobin
	rFel d 4	Lipocalin

(*Continued*)

Table 1. Continued.

Allergen source	Allergen component	Protein group
Mouse	nMus m 1	Lipocalin
Mould		
Alternaria	rAlt a 1	Acidic glycoprotein
	rAlt a 6	Enolase
Aspergillus	rAsp f 1	Mitogillin family
	rAsp f 3	Peroxisomal protein
	rAsp f 6	Mn superoxide dismutase
Cladosporium	rCla h 8	Mannitol dehydrogenase
Mite		
House dust mite	rBlo t 5	Mite group 5
	nDer f 1	Cysteine protease
	rDer f 2	NPC2 protease
	nDer *p* 1	Cysteine protease
	rDer *p* 2	NPC2 family
Storage mite	rLep d 2	NPC2 family
Cockroach		
Cockroach	rBla g 1	Cockroach group 1
	rBla g 2	Aspartic protease
	rBla g 5	Glutathione S-transferase
Other components		
Venom		
Honey bee venom	rApi m 1	Phospholipase A2
	nApi m 4	Melittin
Paper wasp venom	rPol d 5	Venom, Antigen 5
Common wasp venom	rVes v 5	Venom, Antigen 5
Parasite		
Anisakis	rAni s 1	Serine protease inhibitor
Latex		
Latex	rHev b 1	Rubber elongation factor
	rHev b 3	Small rubber particle protein
	rHev b 5	Acidic protein
	rHev b 6.01	Prohevein
Cross-reactive components		
Serum albumin		
Cow's milk/meat	nBos d 6	Serum albumin
Dog	nCan f 3	Serum albumin
Horse	nEqu c 3	Serum albumin
Cat	nFel d 2	Serum albumin
Tropomyosin		
Anisakis	rAni s 3	Tropomyosin
Cockroach	nBla g 7	Tropomyosin
House dust mite	rDer *p* 10	Tropomyosin
Shrimp	nPen m 1	Tropomyosin
Lipid transfer protein (nsLTP)		
Peanut	rAra h 9	Lipid transfer protein (nsLTP)
Hazelnut	rCor a 8	Lipid transfer protein (nsLTP)
Walnut	nJug r 3	Lipid transfer protein (nsLTP)
Peach	rPru *p* 3	Lipid transfer protein (nsLTP)
Mugwort	nArt v 3	Lipid transfer protein (nsLTP)
Olive pollen	nOle e 7	Lipid transfer protein (nsLTP)
Plane tree	rPla a 3	Lipid transfer protein (nsLTP)
PR-10 protein		
Birch	rBet v 1	PR-10 protein
Alder	rAln g 1	PR-10 protein
Hazel pollen	rCor a 1.0101	PR-10 protein
Hazelnut	rCor a 1.0401	PR-10 protein
Apple	rMal d 1	PR-10 protein
Peach	rPru *p* 1	PR-10 protein
Soybean	rGly m 4	PR-10 protein
Peanut	rAra h 8	PR-10 protein
Kiwi	rAct d 8	PR-10 protein

(*Continued*)

Table 1. Continued.

Allergen source	Allergen component	Protein group
Celery	rApi g 1	PR-10 protein
Thaumatine-like protein		
Kiwi	nAct d 2	Thaumatine-like protein
Profilin		
Birch	rBet v 2	Profilin
Latex	rHev b 8	Profilin
Annual mercury	rMer a 1	Profilin
Timothy grass	rPhl *p* 12	Profilin
CCD		
Sugar epitope from Bromelain	nMUXF3	Cross-reactive Carbohydrate Determinants
Polcalcin (Calcium binding 2-EF-hand protein)		
Birch	rBet v 4	Polcalcin
Timothy grass	rPhl *p* 7	Polcalcin

Notes: ImmunoCAP ISAC – Phadia, Thermo Fisher Scientific, Uppsala, Sweden.

Results

Patients

60 patients were examined, 31 men and 29 women with the average age 41.4 years and with the average SCORAD 39.2, s.d.13.1 points. The sensitisation to all tested molecular fungal allergen was recorded altogether in 35 patients (58%). The sensitisation to Alt a 1 was recorded in 16 patients (26%), to Alt a 6 in 11 patients (18%), to Cla h 8 in 2 patients (3%), to Asp f 1 in 5 patients (8%), to Asp f 3 in 5 (8%) patients and to Asp f 6 in 16 patients (26%). The sensitisation to both Alternaria, Aspergilus and Cladosporium was recorded in 10 patients (29%). The characteristics of patients are recorded in Table 2.

We evaluated, if patients suffering from sensitisation to fungal molecullar allergens suffer significantly more from sensitisation to other food and inhallant molecular allergens. The relation between the sensitisation to molecular allergens (Alt a 1, Alt a 6, Asp f 1, Asp f 3, Asp f 6) and sensitisation to different food and inhalant allergens is shown in Table 3. The significant relation is marked with *.

Alt a 1: The significant relation between the sensitisation to molecular allergen Alt a 1 and Cyn d 1 (Cynodon Dactylon), Phl p 4, Phl p 6 (Timothy – Phleum pratense), Act d 2 (Actinidia deliciosa – Kiwi), Hev b 8 (Latex – Hevea brasiliensis) was confirmed.

Alt a 6: The significant relation between the sensitisation to to molecular allergen Alt a 6 and Phl p 4 (Timothy – Phleum pratense), Cup a 1 (Arizona Cypress- Cupressus arizonica), Cry j 1 (Cedar pollen allergen – Cryptomeria), Cor a 1.0101 (Hazel Nut – Coryllus avellana), Pla a 2 (London Plane tree – Platanus acerifolia), Ole e 1, Ole e 9 (Olive- Olea Europaea), Pla l 1 (Ribwort – Plantago Lanceolata), Cla h 8, Der f 2, Der p 2 (European House Dust mite – Dermatophagoides pteronyssinus), Fel d 4 (Cat – Felis domesticus), Mal d 1 (Apple – Mallus domestica), Act d 1 (Actinidia deliciosa – Kiwi), Pru p 1

Table 2. The characteristic of patients with atopic dermatitis.

– 60 patients examined – 31 men, 29 women, the average age 41.4 years
– the average SCORAD 39.2, s.d. 13.1 points
– the sensitisation to all tested molecular fungal allergen – 35 patients (58%)
– the sensitisation to Alt a 1 – 16 patients (26%), Alt a – 6 in 11 patients (18%), Cla h 8 – 2 patients (3%)
– the sensitisation to Asp f 1 – 5 patients (8%), Asp f 3 – 5 patients (8%), Asp f 6 – 16 patients (26%).
– The sensitisation to Alternaria alternata and/or Cladosporium herbarum and/or Apergilus fumigatus – 10 patients (29%).

Table 3. The relation between the sensitisation to molecular fungal allergens (Alt a 1, Alt a 6, Asp f 1, Asp f 3, Asp f 6, Cla h 8), and different food and inhalant allergens according to the molecular component diagnostics Multiplex ISAC testing.

	rAlta1	rAlta6	rAspf1	rAspf3	rAspf6	rClah8
Mollecular allergen			*p* – value			
nCynd1	**0.007***	0.031	0.199	0.199	0.128	0.171
rPhlp1	0.016	0.146	0.403	0.896	0.234	0.267
rPhlp2	0.250	0.147	0.453	0.453	0.976	0.105
rPhlp4	0.226	**0.010***	0.257	0.257	0.072	0.202
rPhlp5	**0.004***	0.034	0.091	0.453	0.082	0.863
rPhlp6	**0.001***	0.047	0.108	0.504	0.115	0.903
rPhlp11	0.990	0.367	0.263	0.200	0.444	0.491
rPhlp7	0.084	0.935	0.477	**0.008***	0.708	0.662
rPhlp12	0.114	0.503	0.589	0.589	0.278	**0.003***
rAlng1	0.024	0.023	0.669	0.022	0.275	0.157
nCupa1	0.318	**0.005***	0.391	0.042	0.318	0.090
nCryj1	0.117	**0.001***	0.660	**0.002***	0.477	0.126
rCora1.0101	0.011	**0.001***	0.227	0.032	0.437	0.186
rPlaa2	0.129	**0.008***	0.838	0.838	0.027	**0.010***
rPlaa3	0.114	0.028	0.589	0.589	0.804	**0.003***
nOLee1	0.129	**0.001***	0.935	0.013	0.129	**0.003***
nOlee9	0.204	**0.002***	0.984	0.984	0.204	0.289
rBetv 1	0.135	0.018	0.325	0.053	0.762	0.233
rBetv2	0.015	0.141	0.660	0.071	0.317	0.126
rBetv 4	0.018	0.247	0.662	0.662	0.459	0.788
rchea1	0.115	0.896	0.432	**0.000***	0.183	0.628
rArtv 1	0.575	0.796	0.121	0.134	0.477	0.542
rArtv 3	0.804	0.028	0.589	0.113	**0.004***	0.739
rAmba1	0.921	0.735	0.528	0.219	0.286	0.698
rPlal1	0.183	**0.001***	0.447	**0.000***	0.718	0.058
rMera1	**0.001***	0.312	0.849	0.151	0.182	0.205
nSalk 1	0.538	0.035	0.759	0.759	0.538	0.850
rparj2	0.885	0.014	0.355	0.660	0.885	**0.000***
rAlta1	rAlta1	0.129	0.708	0.708	0.823	0.459
rAlta6	0.129	rAlta6	0.200	0.200	0.023	**0.003***
rAspf1	0.708	0.200	rAspf1	0.333	0.708	0.662
rAspf3	0.708	0.200	0.333	rAspf3	0.498	0.662
rAspf6	0.823	0.023	0.708	0.498	rAspf6	0.459
rClah8	0.459	**0.003***	0.662	0.662	0.459	rClah8
rDerf1	0.177	0.013	0.017	0.542	0.247	0.584
Derf2	0.728	**0.000***	0.199	0.199	0.035	0.171
rBlot5	0.718	0.037	0.447	0.021	0.718	0.628
rDerp1	0.134	0.021	0.023	0.493	0.329	0.625
rDerp2	0.728	**0.000***	0.199	0.199	0.035	0.171
rDerp10	0.206	0.735	0.219	0.219	0.206	0.698
rBlag1	0.538	0.035	0.759	0.759	0.098	0.850
rBlag2	0.459	0.491	0.662	0.662	0.380	0.788
rBlag5	0.278	0.503	0.589	0.113	0.114	0.739
rBlag7	0.206	0.735	0.219	0.219	0.206	0.698
rlepd2	0.063	0.620	0.071	0.071	0.885	0.569
rFeld1	0.012	0.114	0.404	0.404	0.188	0.093
rFeld2	0.154	0.935	0.477	0.333	**0.005***	0.662
rFeld4	0.122	**0.005***	0.564	0.564	**0.005***	0.024
rCanF1	0.234	0.944	0.272	0.272	0.015	0.705
rcanf2	0.578	0.441	0.151	0.151	0.822	0.516
nCanf3	0.154	0.935	0.477	0.333	0.084	0.662
rCanf5	0.378	0.444	0.708	0.498	0.080	0.380
rEquc1	0.369	0.037	0.564	0.108	0.028	0.024
nEquc3	0.804	0.028	0.589	0.589	**0.004***	0.739
nMusm1	0.587	0.526	0.021	**0.001***	0.587	0.289
rVesv 5	0.718	0.037	0.432	0.432	0.718	0.058
r/nApim1	0.538	0.035	0.759	0.759	0.538	0.850

(*Continued*)

Table 3. Continued.

	rAlta1	rAlta6	rAspf1	rAspf3	rAspf6	rClah8
Mollecular allergen			*p* – value			
rPold5	0.206	0.095	0.528	0.219	0.286	0.698
nSesi1	0.804	0.503	0.113	0.113	0.804	0.739
nTriaaA	0.380	0.247	0.032	0.662	0.380	0.788
nGald1	0.718	0.037	0.447	0.432	0.183	0.058
ngald2	0.708	0.263	0.333	0.333	0.498	0.662
nGald3	0.718	0.037	0.447	0.447	0.718	0.058
nGald5	0.380	0.491	0.032	0.662	0.459	0.788
nBosd5	0.538	0.629	**0.001***	0.759	0.538	0.850
nBosd8	0.538	0.035	0.759	0.759	0.098	**0.000***
nBosd	0.459	0.247	0.662	0.032	0.018	0.788
rMald1	0.226	**0.010***	0.257	0.038	0.535	0.202
nActd1	0.927	**0.005***	**0.001***	0.042	0.927	0.090
nActd2	**0.000***	0.415	0.935	0.935	0.990	0.247
nActd8	0.080	0.444	0.708	0.708	0.274	0.459
rPrup1	0.226	**0.010***	0.257	0.038	0.535	0.202
n/rPrup3	0.498	0.200	0.477	0.333	0.708	0.032
rAnao2	0.804	0.503	0.113	0.589	0.278	0.739
rCora10401	0.211	**0.002***	0.612	0.149	0.506	0.143
rCora8	0.018	0.247	0.662	0.662	0.380	**0.000***
nCora9	0.538	0.035	0.759	0.759	0.098	**0.000***
nArah1	0.804	0.028	0.589	0.113	0.114	**0.003***
nArah2	0.459	0.247	0.662	0.662	0.459	0.788
nArah3	0.459	0.247	0.662	0.662	0.018	0.788
nArah6	0.098	0.035	0.759	0.759	0.098	0.850
rArah8	0.211	**0.002***	0.612	0.149	0.211	0.143
rArah9	0.286	**0.003***	0.219	0.528	0.286	0.013
rGlym4	0.770	0.016	0.061	0.061	0.137	0.082
rGlym5	0.380	0.247	0.032	0.662	0.380	0.788
nJugr1	0.538	0.629	0.759	0.759	0.538	0.850
nJugr2	0.804	**0.000***	0.589	0.113	0.114	0.739
nJugr3	0.286	0.095	0.528	0.219	0.286	0.013
rAnis 1	0.538	0.035	0.759	0.759	0.538	0.850
rAnis 3	0.278	0.395	0.113	0.113	0.278	0.739
rGadc1	0.026	**0.003***	0.219	0.528	**0.001***	0.698
nPenm1	0.718	0.330	0.021	0.021	0.543	0.628
nPenm2	0.801	**0.005***	**0.008***	0.108	0.122	0.501
nPenm4	0.380	0.491	0.662	0.662	0.459	0.788
nBosd6	0.380	0.247	0.662	0.662	0.018	0.788
rApig1	0.531	**0.001***	0.771	0.434	0.049	0.014
rHevb1	0.459	0.247	0.662	0.662	0.380	0.788
rHevb3	0.459	0.247	0.662	0.662	0.380	0.788
rHevb5	0.538	0.629	0.759	0.759	0.538	0.850
hevb6.01	0.498	0.013	0.333	0.333	0.498	0.662
nHevb8	**0.001***	0.057	0.849	0.151	0.182	0.205
nMUXF3	0.129	**0.001***	0.371	0.371	0.129	0.422
	rAlta1	rAlta6	rAspf1	rAspf3	rAspf6	rClah8

The significant relation is marked with *.

(Peach – Prunus persica), Cor a 10401 (Hazel Nut – Coryllus avellana), Ara h 8, Ara h 9 (Peanut – Arachis hypogea), Jug r 2 (Walnut – Juglans regia), Gad c 1 (Atlantic Cod – Gadus), Pen m 2 (Black Tiger Shrimp – Penaeus monodon) and Api g 1 (Cellery – Apium graveolens) was confirmed.

Cla h 8: The significant relation between the sensitisation to to molecular allergen Cla h 8 and Phl p 12 (Timothy – Phleum pratense), Pla a 2, Pla a 3 (London Plane tree – Platanus acerifolia), Ole e 1 (Olive – Olea Europaea), Par j 2 (Wall Pellitory – Parietaria judaica), Bos d 8 (Beef – Bos domesticus), Cor a 8, Cor a 9 (Hazel Nut – Coryllus avellana) and Ara h 1 (Peanut – Arachis hypogea) was confirmed.

Asp f 1: The significant relation between the sensitisation to to molecular allergen Asp f 1 and Bos d 5 (Beef – Bos domesticus), Act d 1 (Actinidia deliciosa – Kiwi) and Pen m 2 (Black Tiger Shrimp – penaeus monodon) was confirmed.

Asp f 3: The significant relation between the sensitisation to to molecular allergen Asp f 3 and Phl p 7 (Timothy – Phleum pratense), Cry j 1 (Cedar pollen allergen – Cryptomeria), Che a 1 (Lamb ´s quarter – Chenopodium album), Pla l 1(London Plane tree – Platanus acerifolia), and Mus m 1 (Mouse – Mus musculus) was confirmed.

Asp f 6: The significant relation between the sensitisation to to molecular allergen Asp f 6 and Art v 3 (Mugwort – Artemisia vulgaris), Fel d 2, Fel d 4 (Cat – Felis domesticus), Equ c 3 (Horse – Equus caballus), and Gad c 1 (Atlantic Cod – Gadus) was confirmed.

The confirmed significant relation between the sensitisation to Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6 and food and inhalant molecular allergens is shown in Table 4.

The level of sIgE (in ISU-E) to molecular allergens (Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6) in 35 patients with confirmed sensitisation to fungi is shown in Table 5.

Discussion

This study shows the sensitisation to molecular fungal allergens in atopic dermatitis patients and confirmes, that patients suffering from sensitisation to different fungal allergens are more often sensitised to some food and inhalant allergens according to CRD examination. The evaluation of sensitisation to outdoor and indoor fungi in atopic dermatitis patients according to the results of molecular component diagnostic has been the subject of only limited studies.

Although the air we breathe contains thousands of mould spores, sensitisation rates for both indoor (e. g., *Aspergillus, Penicillium*) and outdoor fungal species (e. g., *Cladosporium, Alternaria*) is below 5%; however, this rate is subject to regional variation (Haftenberger et al., 2013; Heinzerling et al., 2009; O'Driscoll et al., 2009; Schmitz, Ellert, Kalcklösch, Dahm, & Thamm, 2013). Moulds present no real hazard to the majority of the population, except in the case of high exposure, e. g., resulting from massive distribution. The situation is different for risk groups such as allergics (patients suffering from atopic dermatitis and/or rhinitis) or asthmatics. *Alternaria alternata* (=*Alternaria tenuis*), which is classified as an airborne outdoor mould in the northern hemisphere, appears to be particularly relevant in the development and severity of asthma (O'Driscoll et al., 2009). In addition to allergic rhinoconjunctivitis and allergic asthma, moulds can also induce hypersensitivity pneumonitis (dominated by the antigen-IgG complexes).

Table 4. The confirmed significant relation between the sensitisation to Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6 and food and inhalant molecular allergens.

Allergen	Number of patients	Sensitisation confirmed to molecular allergens
Alt a 1	16	Cyn d 1, Phl p 5, Phl p 6, Mer a 1, Act d 2, Hev b 8
Alt a 6	11	Phl p 4, Cup a 1, Cry j 1, Cor a 1.0101, Pla a 2, Ole e 1, Ole e 9, Pla l 1, Cla h 8, Der f 2, Der p 2, Fel d 4, mal d 1, Act d 1, Pru p 1, Cor a 10401, Ara h 8, Ara h 9, Jug r 2, Gad c 1, Pen m 2, Api g 1, MUXf 3
Cla h 8	2	Phl p 12, Pla a 2, Pla a 3, Ole e 1, Par j 2, Bos d 8, Cor a 8, Cor a 9, Ara h 1, Alt a 6
Asp f 1	5	Bos d 5, Act d 1, Pen m 2
Asp f 3	5	Phl p 7, Cry j 1, Che a 1, Pla l 1, Mus m 1
Asp f 6	16	Art v 3, Fel d 2, Fel d 4, Equ c 3, Gad c 1

Table 5. Patients with confirmed sensitisation to fungi – the level of sIgE to molecular allergens in ISU-E.

Patients	r Alt a 1	r Alt a 6	r Asp f 1	r Asp f 3	r Asp f 6	r Cla h 8
1				2.9	6.7	
2	14					
3	38					
4				15		
5	42	28				
6	16					
7	24					
8			0.4			
9	43					
10	>100	17				6.4
11	4.1					
12		8			20	
13					2.6	
14			4.4			
15					27	
16	16				0.4	
17				1.7		
18	19	9.9	4.8	5.2		
19		0.5	0.8		18	
20					0.6	
21		0.5				
22		27			8	1.5
23	1.1	4.2			7.7	
24		0.4		1.5	2.1	
25					14	
26	1.8					
27	23				6.6	
28	78					
29					8.9	
30	9.2					
31		2				
32					3	
33	50	8.7			4.1	
34					13	
35			0.7			

Notes: Sensitisation to Alternaria alternata: 23 patients – 37.3%.
Sensitisation to Aspergilus fumigatus: 22 patients – 36.6%.
Sensitisation to Cladosporium herbarum: 2 patients – 3.3%.

Despite the numerous mould allergens described to date, only eight single allergens from the three genera Alternaria alternata, Aspergillus fumigatus and Cladosporium herbarum are currently available for molecular diagnostic methods (Chruszcz et al., 2012; Kurup et al., 2000).

In our previous studies we confirmed, that sensitisation to fungi (according to the examination with extract sIgE and skin prick test) was recorded in 30% patients suffering from atopic dermatitis (Čelakovská, Bukač, Ettler, Vaneckova, Ettlerova, et al., 2018; Čelakovská, Bukač, Ettler, Vaneckova, Krcmova, et al., 2018).

At this study, we evaluated the sensitisation to Alt a 1, Alt a 6, Cla h 8, Asp f 1, Asp f 3, Asp f 6 in atopic dermatitis patients. Altogether, the sensitisation to fungi according to CRD examination was confirmed in 35 patients (58%). We confirmed, that atopic dermatitis patients suffering from sensitisation to Alternaria and Cladosporium suffer significantly more from sensitisation to Peanut, Walnut and Hazelnut; it is in agreement with our previous results (Čelakovská, Bukač, Ettler, Vaneckova, Ettlerova, et al., 2018; Čelakovská, Bukač, Ettler, Vaneckova, Krcmova, et al., 2018). Patients suffering from

sensitisation to molecular allergen Alt a 1 suffer significantly more often from the sensitisation to molecular allergens of Cynodon Dactylon, Timothy, Kiwi and Latex. Sensitisation to Alt a 6 is connected with sensitisation to more inhallant and food allergens, such as Apple, Kiwi, Peach and Celery, Timothy, London Plane tree and Olive. Sensitisation to Aspergilus is connected with sensitisation to some inhalant allergens, such as Timothy, Cedar pollen allergen, Lamb ´s quarter, London Plane tree, Mugwort, Mouse, Cat, Horse and to some food allergens, such as Atlantic Cod and Black Tiger Shrimp. Our previus results confirmed, that atopic dermatitis patients suffering from food hypersensitivity reactions to sea fish and seafood suffer significantly more from sensitisation to fungi (Čelakovská, Bukač, Ettler, Vaneckova, Krcmova, et al., 2018; Čelakovská, Bukač, Ettler, Vaneckova, Krcmova, et al., 2018).

Sensitisation to Alternaria alternata spores are considered a well-known biological contaminant and a very common potent aeroallergen source that is found in environmental samples. At our study, sensitisation to Alt 1 and Alt 6 was alogether confirmed in 23 patients (37%). The most intense exposure to Alternaria alternata allergens is likely to occur outdoors; however, Alternaria and other allergenic fungi can colonise in indoor environments and thereby increase the fungal aeroallergen exposure levels. Among allergenic proteins described in this fungal specie, the major allergen, Alt a 1, has been reported as the main elicitor of airborne allergies in patients affected by a mould allergy and considered a marker of primary sensitisation to Alternaria alternata. Moreover, Alternaria alternata sensitisation seems to be a triggering factor in the development of poly-sensitisation, most likely because of the capability of Alternaria alternata to produce, in addition to Alt a 1, a broad and complex array of cross-reactive allergens that present homologs in several other allergenic sources. The study and understanding of Alternaria alternata allergen information may be the key to explaining why sensitisation to Alternaria alternata is a risk factor for asthma and also why the severity of asthma is associated to this mould. Recent research on the identification and characterisation of Alternaria alternata allergens has allowed for the consideration of new perspectives in the categorisation of allergenic moulds, assessment of exposure and diagnosis of fungi-induced allergies (Gabriel, Postigo, Tomaz, & Martínez, 2016). The sensitisation to Asp f 1, Asp f 3 and Asp f 6 was altogether confimed on 22 patients (36%). *Aspergillus fumigatus* as a ubiquitous fungus can be found in the respiratory tract of the asthmatic and healthy people (Overton, Simpson, Bowyer, & Denning, 2017; Woolnough et al., 2017). The inhalation of *Aspergillus* spores leads to an immune response in individuals. The evaluation of serum specific IgE and IgG against *Aspergillus fumigatus* is considered as the main criteria for the diagnosis of allergic diseases due to *Aspergillus* (Singh, Paul, Singh, & Nayak, 2018). The relation between the sensitisation to fungi and other inhallant allergens was confirmed in our study. The explanation is in the nature of outdoor allergen-bearing particles, the distributions of their source, and the nature of the aerosols (particle types, sizes, dynamics of concentrations). Primary sources for outdoor allergens include vascular plants (pollen, fern spores, soy dust), and fungi (spores, hyphae) (Burge & Rogers, 2000). Nonvascular plants, algae, and arthropods contribute small numbers of allergen-bearing particles. No official standards exist for interpretation of pollen or fungal data. The American Academy of Asthma, Allergy and Immunology published guidelines for interpretation of pollen data that are based on national averages for groups of pollen types. Outdoor fungal aerosol concentrations are also listed. Whether these guidelines relate to a

disease in any way remains unknown (Burge, 2002). Jariwala et al. performed the study to better understand the contribution of pollen and fungi to asthma severity in Bronx. According to their results, there exists a significant association between spring asthma bronchiale and tree pollen concentrations in a highly urbanised area such as Bronx (Jariwala et al., 2014; Segura et al., 2016).

The prevalence of fungal sensitisation displays wide geographical variability. Data from the European Community Respiratory Health Survey demonstrated that among adults aged 20–44 years in the general population, the prevalence of positive skin tests using *Alternaria* and *Cladosporium* extracts ranged from 0.2% to 14.4%, and 0–11.9%, respectively (Bousquet et al., 2007; Chou et al., 2003; Fairs et al., 2010; Wenzel, 2006). At our study, positive results according to CRD examination to fungal molecular allergens were obtained in 58% of atopic dermatitis patients. The explanation is, that we evaluate this sensitisation in patients suffering from atopic dermatitis and the incidence of sensitisation to fungi can be higher in this group of patients suffering from atopic disease (Bousquet et al., 2007; Chou et al., 2003; Fairs et al., 2010; Wenzel, 2006). According to Crameri, the incidence of fungal sensitisation is high and clinically relevant. However, the problems related to the *in vitro* and *in vivo* diagnosis of fungal and other allergies are far from being solved (Crameri, Garbani, & Rhyner, 2014). Any *in vivo* diagnosis of allergy based on skin tests as well as any *in vitro* diagnosis of allergy based on the determination of allergen-specific IgE depends on the quality of the material used for testing (American Academy of Allergy. Asthma and Immunology (AAAAI), 1997; Slater et al., 2012).

Nolles et al. investigated the prevalence of sensitisation to different fungi in atopic children in relation to age and other aeroallergens. Specific IgE for indoor and outdoor fungi was associated with the presence of specific IgE for aeroallergen and milk. The conclusion of the study is that sensitisation to fungi is prevalent in childhood with an age-dependent distribution reaching maximum values at 7.7–7.8 years, followed by a decline for all fungal sensitisation with increasing age (Nolles, Hoekstra, Schouten, Gerritsen, & Kauffman, 2001). Reijula et al. evaluated the prevalence of IgE-mediated allergy and clinical outcomes caused by sensitisation to fungal allergens in patients with suspected allergy. The conclusion of the study is, that in the Finnish population with allergic symptoms, IgE-mediated sensitisation to 2 common fungal allergens was rare and of minor clinical importance. Positive skin prick test reactions to fungi are mostly observed in patients with multiple sensitivity to various allergens (Reijula et al., 2003). The aim of another study was the analysis of specific IgE against *Alternaria alternata* in atopic dermatitis and asthma patients. A total of 50 AD patients (male 17 and female 33) and 50 asthma patients (male 20 and female 30) were entered in the study (Hedayati, Arabzadehmoghadam, & Hajheydari, 2009). This study suggests that *Alternaria alternata* is a major aeroallergen. Some previous studies have shown that *Alternaria alternata* is one of the most common indoor and outdoor airborne fungi, so it could permanently present some allergens to susceptible individuals. Therefore, control of *Alternaria alternata* growth in indoor areas and avoidance with *Alternaria alternata* could play an important role in reducing allergic reaction in susceptible individuals (Hedayati et al., 2009).

In our previous studies we evaluated the occurrence of food allergy and food hypersensitivity reactions in patients suffering from atopic dermatitis (Celakovska, Ettlerova, Ettler, Vanecková, & Bukac, 2015; Čelakovská, Krčmová, Bukač, & Vaněčková, 2017; Čelakovská, Bukač, Ettler, Ettlerova, & Krcmova, 2017; Čelakovská & Bukač, 2017a;

Čelakovská & Bukač, 2017b). The food hypersensitivity reactions were recorded 83% of patients, the most often reactions were recorded after ingestion of nuts, tomatoes, kiwi, apples, spices, oranges and lemons, fishes, capsidum, celery and carrots. Patients suffering from food hypersensitivity reactions in generall suffer significantly more often from rhinitis and from persistent eczematic lesions; there is a significant dependence between patients suffering from food hypersensitivity to nuts and the occurence of rhinitis and asthma bronchiale; another significant relation was confirmed in patients suffering from food hypersensitivity to kiwi and fishes to the duration of eczematic lesions and in patients suffering from food hypersensitivity to apple to the occurence of rhinitis (Celakovska et al., 2015; Čelakovská, Krčmová, et al., 2017; Čelakovská, Bukač, et al., 2017; Čelakovská & Bukač, 2017a; Čelakovská & Bukač, 2017b).

The sens of this study was to show the sensitisation to molecular fungal allergens in patients suffering from atopic dermatitis and to evaluate, if patients suffering from this sensitisation suffer more often from sensitisation to some food and inhallant molecular allergens. We confirmed the high occurrence of sensitisation to molecular fungal allergens in atopic dermatitis patients and the relation to some other food and inhallant allergens.

Conclusion

The sensitisation to all tested molecular fungal allergens was recorded altogether in 58% of atopic dermatitis patients. Sensitisation to molecular fungal allergens is in significant relation to sensitisation to some food and inhallant molecular allergens, such as Peanut, Walnut, Hazelnut, Atlantic Cod and Black Tiger Shrimp, Apple, Kiwi, Peach, Celery, Timothy, London Plane tree, Cedar pollen allergen, Mugwort, Mouse, Cat, and Horse.

Disclosure statement

No potential conflict of interest was reported by the authors.

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