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Single Nucleotide Polymorphism of Dectin-1 Gene Associates with Atopic Dermatitis in Children

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Abstract

BACKGROUND: Atopic dermatitis (AD) is a chronic inflammatory skin disease with complex and multifactorial pathophysiology, involving elements of barrier dysfunction, alterations in cell-mediated immune responses, IgE sensitization, and environmental factors. This encourages the search for predictors of disease development among both genetic markers and environment.

AIM: The aim of the study was to examine if genetic factors of Malassezia recognition, or Malassezia colonization may be related to IgE sensitization or to severity of AD.

METHODS: The study included 106 patients with eczema and 103 healthy children. Specific IgE against *Malassezia* mix (m227) was analyzed in 51 patients using immunochemiluminescent method on the ImmunoCAP 100 (Thermo Fisher Scientific Inc., Phadia, Sweden). Genotyping for rs7309123 in Dectin-1 was performed using Real-time PCR. The level of colonization by *Malassezia* in the scale samples was determined by a real-time PCR assay.

RESULTS: Increased IgE to *Malassezia* spp. was observed in 29,4% of children with eczema. Higher *Malassezia* spp. – specific IgE titer positively correlated with severity of AD, age of onset, head–neck type of AD, and a higher total IgE. GG genotype rs7309123 Dectin-1 is significantly more often found in the patients than in the control group, but no correlation with IgE sensitization to *Malassezia* was found. *Malassezia restricta* and *M. globosa* were predominant in patients and controls, with some predominance of *M. globosa* over *M. restricta* among patients.

CONCLUSION: Sensitization to *Malassezia*, genetic markers in Dectin-1, and *Malassezia* colonization of the skin can be tools for studying the gene-environment interactions in the pathogenesis of AD.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease with a typical characteristic of eczematous lesions. Fungi usually colonize on the skin of AD patients, but the role of these fungi in the pathogenesis of AD remains unclear. The opportunistic yeast *Malassezia* belongs to the normal cutaneous flora. *Malassezia* can also cause IgE-mediated sensitization in patients experiencing AD [1]. IgE, specific for *Malassezia* spp., was found in 5–27% of children [2], [3], [4] and 29–65% of adults with AD [5], [6], [7], [8].

C-type lectin receptor family members are specialized in sensing fungal microbes and linking fungal recognition to the initiation of T-cell responses, in particular those of the Th17 type. Several human studies support the idea that the interaction of Dectin-1 with its ligand promotes the Th2 environment either through instructing naive T-cells, followed by the release of Th2-type cytokines [9], or the release of chemokines (CCL20) associated with a set of Th2 cells [10]. SNP rs7309123 in Dectin-1 showed the strongest association with risk of invasive pulmonary aspergillosis infection [11].

The aim of this study was to identify novel clinical and genetic risk factors for severe AD, accompanied by sensibilization to *Malassezia* yeasts. We hypothesized that polymorphism rs7309123 in intron region of Dectin-1 could be associated with childhood eczema and sensibilization to *Malassezia* in children with AD. To test this hypothesis, we examined differences in the frequency of this SNP between healthy controls and children with eczema, and the association of polymorphism rs7309123 with sIgE to *Malassezia* yeasts.

Materials and Methods

The study included patients with AD (n = 106), aged 6 month–18 years (6 [3;10]) from the Department of Allergy at Kyiv City Children Clinical Hospital №2 and 103 children aged 12 month–18 years (6 [5;9]) without the allergic diseases. This study was approved by the Ethical Committee of the O. Bogomolets National Medical University, all patients/parents of the children gave an informed consent to participate.

The diagnosis of AD was established according to the U.K. working party's diagnostic criteria [12], by the patient's history. Clinical parameters of patients included age, gender, age of onset and severity of eczema, AD distribution (head–neck type eczema vs. diffuse AD without head and neck involvement), concomitant allergic diseases, parental history of atopy, total IgE, and sIgE to *Malassezia*. The severity of AD was assessed using SCORing AD (SCORAD) index [13].

Testing of IgE antibodies

Specific IgE to (m227), a mixture of 3 *Malassezia* species (*Malassezia sympodialis*, *Malassezia globosa*, and *Malassezia restricta*), was analyzed in 51 patients. Serum samples from patients were collected during visits and stored at – 20°C before analysis. All samples were analyzed together for the presence of total IgE antibodies and allergen specific IgE antibodies to *Malassezia* spp. (m227) using immunochemiluminescent method on the ImmunoCAP 100 (Thermo Fisher Scientific Inc., Phadia, Sweden). Class 1 or higher was defined as positive. The results of specific IgE to *Malassezia* were matched with patient-related parameters (gender, age, head–neck type eczema, SCORAD, age of onset, and total IgE).

Selection of SNP

An SNP rs7309123 in Dectin-1, located in intron region, was selected for genotyping, as it can affect expression of Dectin-1 and is reported to be common in European populations.

DNA extraction

Buccal epithelium was taken using buccal brushes, skin samples were collected from the forearm by swabbing, followed by freezing of the samples and their storage at –20°C. DNA for genotyping was extracted from the samples using NeoPrep 100 DNA (Neogen, Ukraine) according to the manufacturer's protocol. The concentration of total DNA was determined using a NanoDrop spectrophotometer ND1000 (NanoDrop Technologies Inc., USA).

qPCR genotyping

Amplification reactions were performed using a 7500 fast real-time PCR System ("Applied Biosystems," USA) in a final reaction volume of 20 μl, which contained 2X TaqMan Universal Master Mix ("Thermo Scientific," USA), assay C_3130832_10, and the template DNA. The thermal cycling conditions involved a denaturation step at 95°C for 20 s, followed by 40 cycles of amplification at 95°C for 3 s and at 60°C for 30 s. Analysis of the data was carried out with 7500 fast real-time PCR Software.

The level of colonization by *Malassezia* in the scale samples was determined by a real-time PCR assay, using primers as described by Sugita *et al*. [14] Table 1.

Table 1: Primers and probes sequences for real-time polymerase chain reaction assays

Target	Sequence
M. globosa	GlobF 5′-GGCCAAGCGCGCTCT-3′
	GlobR 5′-CCACAACCAAATGCTCTCCTACAG -3′
	GlobP 5′-FAM-ATC ATC AGG CAT AGC ATG -BHQ1
M. furfur	FurF 5`-CTT TGG GAC ACA CTC TGC AA-3′
	FurR 5`-TCA CAA GAA CTG CTC CAT GC-3′
	FurP 5`-HEX-GCC TTT GTC ACT CTG TGG GT-BHQ1
M. restricta	ResF 5`-GGC GGC CAA GCA GTG TTT -3′
	ResR 5`-AAC CAA ACA TTC CTC CTT TAG GTG A-3′
	ResP 5`-HEX-TTC TCC TGG CATGGCAT-BHQ1
M. slooffiae	SlofF 5`-GGG ACA TCG TAG AGG GTG AA-3′
	SlofR 5`-CGC TTC CAT TTC GAC AAT TT-3′
	SlofP 5`-FAM-CAT GGA CGT ACC ATG CTT TG-BHQ1
M. sympodialis	SymF 5`-TAG TGA AAG TTT CGG GCC TG-3′
	SymR 5`-GTA AGG GGA GGG AGA ATT CA-3′
	SymP 5`-HEX-GCG CCC ATC ACT ATA TCC AT-BHQ1
M. pachydermatis	PachyF 5`-GGA AAC TAC AAC AGG CTC GC-3′
	PachyR 5`-CAC CAA CCT ACG CAA CAC AG-3′
	PachyP 5`-FAM-CAC CAC CGG TTA TTC CAA AC-BHQ1
rs7309123 Dectin-1 (CLEC7A) (https://www.ncbi.nlm.nih.gov/SNP/)	CP1:GTAGAAGTATACGTGTTGAAATAATAGATTAC
	GP1:GTAGAAGTATACGTGTTGAAATAATAGATTAG
	C/G P2:ACCTTTCACATATCTTCCGGTCATC

M. globosa: *Malassezia globosa*, *M. furfur*: *Malassezia furfur*, *M. restricta*: *Malassezia restricta*, *M. slooffiae*: *Malassezia slooffiae*, *M. sympodialis*: *Malassezia sympodialis*, *M. pachydermatis*: *Malassezia pachydermatis*.

Statistical analysis

Since the distribution of most of the sample characteristics differed from the Gaussian (normal) distribution, the statistical sample was heterogeneous, and therefore, non-parametric statistical methods were used. Quantitative data for each of the study groups were presented as median – Me[QI; QIII], categorical (dichotomous qualitative) variables – as the frequency of each of the values and the percentage in the group.

Multivariate analysis was made to analyze the possible factors related to AD severity. In the analysis of influence sensitization to *Malassezia* spp. on AD, Mann–Whitney U-test was used to compare differences between groups for quantitative data and Fisher's test was used for categorical variables. Spearman's ranking criterion (ρ) was used to assess the correlation between quantitative traits. $p < 0.05$ was considered statistically significant.

Statistical processing was performed using EZR software version 1.32 (graphical interface R [version 2.13.0]).

SNP Analyzer (web-based software) was used to examine Hardy-Weinberg equilibrium. χ^2 test was performed to investigate if there was any difference in the frequency of the genotype and the allele between the AD patient group and the healthy control group.

Results

One hundred and six children between 6 months and 18 years of age were investigated in our

study. The average age of children in the main group was 6 [3;10] years, including 54 boys and 52 girls. Among them, 80.6 % had moderate-to-severe AD according to SCORAD, 19.4% – mild AD. Other concomitant allergic pathology was determined in 32.2% of children (allergic rhinitis, asthma).

About 77.7% patients had the disease onset up to 18 months of age and 22.3% children had late onset. About 41.0% had parental history of atopy. About 18.9% of patients had the head–neck type AD, nine boys and 11 girls, medium age 8.14 ± 5.4.

Testing of IgE antibodies

Serum IgE to *Malassezia* was evaluated in the group of 51 children. Increased IgE to *Malassezia* spp. was observed in 15 (29.4%) children with AD. Among them, 6 (40%) children had class 1 of sensitization, 1 (6.7%) – had 2nd class, 3 (20.0%) children – had 3rd class, and 5 (33.3%) children – the 4th class. Among children with positive IgE to *Malassezia*, there were nine males and six females ($p > 0.05$).

We have found a strong correlation of sensitization to *Malassezia* spp. with a higher SCORAD in children. The coefficient of the correlation of Spearmen (ρ) was 0.767 ($p < 0.05$). Sensitization to *Malassezia* was more often observed in children with an early phenotype (AD beginning in the age <18 months), $\rho = 0.541$ ($p < 0.05$). Higher *Malassezia* spp. – specific IgE titer positively correlated with a higher total IgE titer, $\rho = 0.697$ ($p < 0.05$). We found significant association of head–neck type of AD with IgE specific to *Malassezia* spp. (OR – 7.77 (95% CI 1.95–30.95), $p < 0.05$) Table 2.

Table 2: Demographic, historical, clinical, and serological data of AD patients, which had been tested for serum IgE to *Malassezia* (n = 51)

Parameters	*Malassezia*-IgE>=0.35 n = 15	*Malassezia*-IgE<0.35 n = 36
Age (years), Me [QI; QIII]	7 [3.5; 11]	7 [3; 9.25]
Duration of AD , Me [QI; QIII]	7 [3; 11]	4 [2; 8.5]
SCORAD, Me [QI; QIII]	55 [30; 60]	35 [30; 60]
Total serum IgE, Me [QI; QIII] **	560 [282.7; 896.25]	120,5 [53.25; 267.25]
Malassezia specific IgE, Me [QI; QIII] **	4,46 [0.45; 13.93]	0,01 [0; 0.06]
Age of onset before 18 mo, n (%)	14 (93.3)	29 (80.6)
Duration of AD>5 years, n (%)	9 (60)	17 (47)
Head–neck type AD, n (%)	8 (15,7)	7 (13,7)
Concomitant, n (%)		
Asthma	6 (40)	8 (22.2)
AR	6 (40)	16 (44.4)
Asthma+AR	3 (20)	7 (19.4)
Gender, n (%)		
Male	9 (60)	18 (50)
Female	6 (40)	18 (50)
Gene, n (%)		
CC	5 (33.3)	7 (19.4)
CG	5 (33.3)	20 (55.6)
GG	5 (33.3)	9 (25)

**A statistically significant result at P<0.05 by the Mann–Whitney U-test.

Genotyping

We found that SNP was in Hardy-Weinberg equilibrium. Our main results are as follows: 26.2% of patients and 44.6% of control group had CC genotype, 52.4% and 39.6% of patients and control group, respectively, were heterozygotes (CG; $p < 0.05$ by χ^2–test, OR – 2.26 (95% CI 1.22–4.25) and 21.4% and 15.8% had GG genotype (GG; $p < 0.05$ by χ^2–test, OR – 2.35 (95% CI 1.08–5.15). GG genotype rs7309123 of Dectin-1 is found significantly more often in the AD group than in the control group Figure 1.

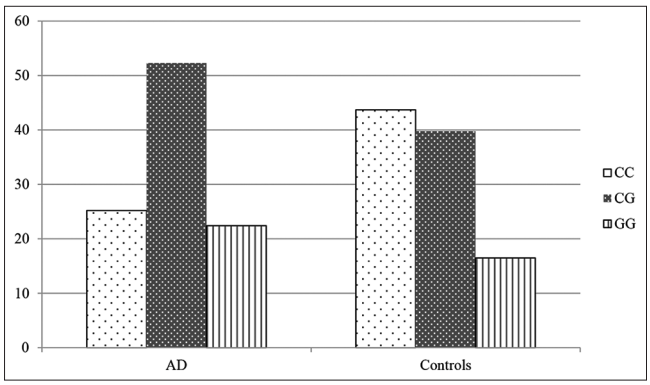

*Figure 1: Genotypic distribution of Dectin-1 gene, comparison between atopic dermatitis and control groups (*p < 0.05)*

GG-genotype was significantly more frequent among patients with increased serum total IgE (OR – 4.129 (95% CI 1.273–13.393)). Patients with GG also were significantly more prone to develop concomitant asthma (OR – 4.800 (95% CI 1.118–20.612). We have not found a relationship between genotype and the presence of sIgE to *Malassezia*, AR, and the age of onset.

A multifactor model revealed that patients with GG-genotype have a higher risk of AD ($p = 0.016$) (OR – 0.46 (95% CI 0.25–0.86), the area under the ROC curve AUC = 0.63 (95% CI 0.56–0.71). Furthermore, multivariate analysis demonstrated, that among other factors, duration of the disease was associated with AD severity, the area under the ROC-curve AUC = 0.78 (95% CI 0.69–0.88), ($p < 0.05$). Thus, patients with a longer duration of the disease are more likely to have a severe course (SCORAD > 50), $p = 0.0002$ (OR – 1.2 (95% CI 1.09–1.33).

M. sympodialis and *M. furfur* were not detected in samples, *M. pachydermatis* and *M. slooffiae* were found in ten patients and one control, so we did not analyze these data. *M. restricta* and *M. globosa* were detected in 11 patients and ten controls. Among children with AD, there were three males and seven females, with medium age of 6 years. We have found significant predominance of *M. globosa* on *M. restricta*, especially among males. Six of them were not sensitized to *Malassezia*, one had positive IgE. Among healthy controls, four males and six females with medium age of 7.5 years, the ratio of *M. globosa* and *M. restricta* was in favor of *M. globosa*, but not as significant as in patients, and, similarly, more prominent among male samples. Thus, the distribution of *Malassezia* by species is likely to be gender-dependent and appears to be dependent on skin conditions such as the presence of AD. However, the number of samples in this study was too limited for reaching definitive conclusions.

Discussion

The incidence of AD among children has increased over the past few decades in Ukraine [15]. AD patients with severe eczema and higher total IgE levels appear to be frequently sensitized to microbial antigens. We assessed the IgE-mediated sensitization to *Malassezia* in 51 children with AD and correlated these data with gender, age, head–neck type eczema, disease severity, age of onset, and total IgE. Our results showed that sensitization to *Malassezia* was prevalent in 29.4% of children with AD. Higher IgE to *Malassezia* spp. was a marker for more severe AD in children. Patient age or gender did not correlate with IgE to *Malassezia* spp. According to the previous studies [7], *Malassezia* spp. – specific IgE was positively correlated with the total serum IgE. Sensitization to *Malassezia* was significantly more often observed in children with an early phenotype of AD. Furthermore, we found significant association of head–neck type of AD with IgE specific to *Malassezia* spp. (OR – 7.77 (95% CI 1.95–30.95), p < 0,05). Reactivity to *Malassezia* allergens was found to be increased in AD patients with head and neck dermatitis in previous studies [16], [17].

Dectin-1 is well known for its role in anti-fungal defense [18]. Chen *et al*. and White PL showed influence of Dectin-1 rs7309123 on the susceptibility to the pulmonary invasive fungal disease in hematology patients (OR – 1.919; [95% CI 1.047–3.518], p = 0.03), (OR – 3.7 [95% CI 1.5–9.3]) [11], [19]. Significant associations of Dectin-1 rs7309123 SNP and fungal infection susceptibility was demonstrated in other studies [20], [21]. Fischer M demonstrated that patients carrying the Dectin-1 SNP rs7309123 G/G (n = 47) or G/G and C/G (n = 133) genotype revealed significantly higher risk for developing pulmonary invasive fungal disease (OR – 2.4, p = 0.041) [22].

We hypothesized that there is a possibility that the misrepresentation of fungi, specifically *Malassezia*, by receptors, due to their polymorphism, could affect the development of sensitization to fungi in AD, and this, in turn, would affect the severity of the disease. The role of polymorphism in the Dectin-1 gene in AD has been studied for the first time. Variants with the GG-genotype of the Dectin-1 rs7309123 were found significantly more frequently among patients than in controls (OR: 2.35 [95% CI 1.08–5.15], p < 0.05), so this polymorphism may have a predictive value in the development of AD. We have not found significant relationship between genotype and IgE sensitization to *Malassezia*, although its development may take place later in adulthood.

A multivariate analysis revealed that patients with GG-genotype have a higher risk of AD (p = 0.016) (OR – 0.46 (95% CI 0.25–0.86), and, among other factors, duration of the disease was associated with AD severity (SCORAD > 50), p = 0.0002 (OR – 1.2 (95% CI 1.09–1.33).

We also hypothesized that an altered ratio of Malassezia species that colonize the skin may be the cause of the development of sensitization. To study this, we compared the ratio of skin colonization in AD and skin of healthy children. Sugita *et al*. showed that the level of Malassezia in the skin of healthy subjects is different between the sexes and changes with age with predominance of *M. restricta* in males in Japanese population [14]. Saad *et al*. analyzed the colonization of the skin with pityriasis versicolor as well as healthy individuals with *M. globosa* and *M. restricta* using PCR and showed that *M. globosa* predominated at lesional sites, while *M. restricta* predominated at non-lesional sites for all tested body sites [23]. In recent study, it was demonstrated that mean ratio of *M. restricta* to *M. globosa* was increased in the AD group than in the control group; however, there were no statistical significance [24].

We have found that distribution of *Malassezia* by species is different among healthy and controls, and, is likely to be gender-dependent: significant predominance of *M. globosa* on *M. restricta* was more prominent among children with AD and in males.

It is extremely important to identify patients, who are at risk of significant deterioration of AD or lack of effectiveness of standard therapy in consequence of sensitization to *Malassezia*. According to the previous studies and our results, these are patients with the head and neck type of AD, patients with genetic variants of pattern recognition receptors, patients with fungal skin dysbiosis, and sensitization to *Malassezia*. Such patients can receive significant benefits from antifungal treatment in the complex treatment of AD.

Conclusion

Our study revealed significant associations between AD and Dectin-1 rs7309123 GG genotype. Furthermore, it identified *Malassezia* spp. – specific IgE as an important allergen-specific marker for AD severity in children. We have also demonstrated that the identification of *Malassezia* species using PCR can serve as a tool for studying the relationship between the skin microbiome and the pathogenesis of AD. Genetic markers in Dectin-1 can be combined with established clinical risk factors in a cohort of high-risk patients to determine whether a management strategy could stratify patients according to the risks, providing personalized medical care to control AD.

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