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ORIGINAL PAPER/*PRACA ORYGINALNA*

House dust mite sensitivity in atopic children with Dectin-1 rs7309123 polymorphism

Uczulenie na roztocze kurzu domowego u dzieci z atopią i polimorfizmem *dectin-1* rs7309123

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ABSTRACT

Introduction: Sensitization to house dust mites is common in patients with atopic dermatitis. Dectin-1 polymorphism can affect the recognition of house dust mite allergens in the skin.

Aim: To investigate the prevalence of sensitization to house dust mites in children with atopic dermatitis and the role of Dectin-1 rs7309123 polymorphism in the development of sensitivity to house dust mites.

Material and methods: The study included 101 patients with atopic dermatitis. Genotyping of the Dectin-1 rs7309123 was performed in the patient group using real-time PCR. Measurements of sIgE to dust mites were performed by Western blotting.

Results: Sensitization to house dust mites was found in 48.5% of the children. The presence of sIgE to HDM in patients with AD was significantly associated with concomitant allergic rhinitis (OR = 2.593 (1.096–6.133)) and in the group of children with both concomitant allergic diseases (OR = 7.432 (1.553–35.564)). There was statistically significant difference in the distribution of genotypes in patients with elevated serum IgE to HDM and among the children with normal levels (OR = 3.111 (0.924–10.480) for CC and GG; OR = 4.267 (1.432–12.716) for CG and GG).

Conclusions: The susceptibility to dust mites among children with atopic dermatitis is 48.5%. Dectin-1 is involved in the development of HDM-induced allergic inflammation in skin because polymorphism rs7309123 of the Dectin-1 gene may affect percutaneous sensitization to HDM in patients with AD, and the inhibition of signalling pathways of Dectin-1 may be an efficient therapeutic strategy.

KEY WORDS

children, house dust mites, atopic dermatitis, dectin-1.

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INTRODUCTION

Atopic dermatitis (AD), the most common skin disease in infants and children, is a complex disease with a multifactorial aetiology, including genetic and environmental factors [1]. Children with AD are at risk of developing respiratory allergies. This risk may be higher for children who develop percutaneous sensitization to house dust mite (HDM) allergens [1]. The relationship between the amount of HDM on the skin and in patients' homes and the severity of AD has long been known [2]. The results of application testing with HDM allergens have shown that it is possible to induce typical skin lesions *in vivo*, especially in places exposed to these air allergens. This is confirmed by the studies of many researchers [3, 4]. Positive application tests showed the presence of immunoglobulin E (IgE)-bearing Langerhans cells in the epidermis of patients with AD [5]. At the same time, the patients develop a delayed type of hypersensitivity reaction due to a severe skin condition, high levels of total IgE, as well as HDM-specific IgE [3]. Violation of the skin barrier in AD leads to increased permeability of the epidermis and the penetration of allergens and irritants through the skin [6]. The key role of HDM in the development of AD is related to the activity of proteolytic enzymes, activation of PAR-2 receptors, and pro-inflammatory activity due to sIgE to HDM [5]. HDM is a carrier not only of allergens, but also of microbial auxiliary compounds, which can stimulate innate signalling pathways and lead to allergy.

House dust mites (HDM), a representative allergen in allergic asthma, have been shown to contain some Dectin-1 ligands, including β-glucan [7], which increases the likelihood of Dectin-1 being involved in the pathogenesis of HDM-induced allergic inflammation of the respiratory tract.

Ito T. investigated the role of Dectin-1 in HDM-induced allergic airway inflammation in Dectin-1-deficient mice (Clec7a-/-) [8]. The authors found that eosinophilic and neutrophilic inflammation, as well as the differentiation of Th2 and Th17, significantly decreased in Clec7a-/- mice. In the lungs, Dectin-1 was expressed on CD11b+ (DC) dendritic cells, which were involved in the induction of allergic airway inflammation [8]. The authors also found that chemokine receptor expression and DC migration were impaired in Clec7a-/- mice. Also, it was demonstrated that dectin-1 signalling plays a key role in the production of prostaglandin E2 (PGE2) in models of asthma induced by HDM [9]. To determine whether dectin-1 is involved in the production of PGE2 macrophages stimulated by HDM, the authors developed bone marrow-derived macrophages (BMDM) from wild-type mice (WT) and mice with dectin-1 deficiency (Clec7a-/-), and analysed PGE2 levels in the supernatants by ELISA after dust mite stimulation. HDM induced the production of PGE2 in BMDM in the WT mice. Importantly, HDM-induced PGE2 production was less significant in Clec7a-/- than in WT, suggesting that Dectin-1 is involved in HDM-induced PGE2 production in macrophages. These results indicate that Dectin-1 plays an important role in the development of HDM-induced allergic airway inflammation by activating CD11b + DC.

AIM

The aim of our study was to examine the prevalence of HDM sensitization in children with AD and the role of the rs7309123 polymorphism in the Dectin-1 gene in its development.

MATERIAL AND METHODS

The study included patients with AD (n = 101) aged 6 month to 18 years (6 (3–10)) from the Department of Allergy at Kyiv City Children's Clinical Hospital No. 2 and 84 children aged 12 months to 18 years (6 (5–9)) without allergic disease at the time of examination or according to the history data. This study was approved by the Ethical Committee of the O. Bogomolets National Medical University, and all patients/parents of the affected children gave informed consent to participate.

OUTCOME MEASURES

The diagnosis of AD was established according to the criteria of Hanifin and Rajka, from the patient's history. Clinical parameters of patients included age, gender, age of onset and severity of eczema, concomitant allergic diseases, and parental history of atopy and sIgE to HDM. Scoring of AD severity and blood sampling to determine IgE levels were performed at the same visit. The severity of AD was assessed using the SCORing Atopic Dermatitis (SCORAD) index. A SCORAD < 25 indicated mild AD, a SCORAD between 25 and 50 indicated moderate AD, and a SCORAD > 50 (with a maximum index of 103) indicated severe AD.

Allergic rhinitis (AR) was diagnosed in 34 patients based on their medical histories and a positive physical examination and the presence of one or more of the following nasal symptoms: rhinorrhoea, nasal congestion, sneezing, and itchy nose for 3 months. Nineteen patients were diagnosed with asthma, which was determined based on the history of the disease, namely the symptoms of wheezing in the last 12 months.

TESTING OF IGE ANTIBODIES

SIgE measurements were performed by Western blotting according to the manufacturer's protocol (Simesta-Medivis, Ukraine-Germany). The results were classified into

the following classes: 0 (less than 0.35 kO/l), 1 (0.35–0.7 kO/l), 2 (0.7–3.5 kO/l), 3 (3.5–17.5 kO/l), 4 (17.5–50 kO/l), 5 (50–100 kO/l), and 6 (100 kO/l). Class 1 or higher was defined as positive.

DNA EXTRACTION

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

QPCR GENOTYPING

Amplification reactions were performed by 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.

STATISTICAL ANALYSIS

Statistical processing was performed using EZR software version 1.32 (graphical interface R version 2.13.0). Because the distribution of most of the sample's 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 a median - Me (QI–QIII) and categorical (dichotomous qualitative) variables - as the frequency of each of the values (n) and the percentage (%) in the group. In the analysis of the relationship of sensitization to HDM spp. and clinical parameters of AD, the odds ratio (OR) was used. A p-value < 0.05 was considered statistically significant. SNPAnalyzer (web-based software) was used to examine the Hardy-Weinberg equilibrium. The χ^2 test and OR 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

In total, 101 children with AD were examined: Sensitization to HDM was detected in 49 (48.5%) children; in 52 (51.5%) patients with AD the result was negative. In the group of children susceptible to dust mites, there were 27 (55.1%) boys and 22 girls (44.9%), the median age was 7 years (3–11). In the group of non-sensitized children there were 26 (50.0%) boys and 26 (50.0%) girls, and the median age was 7.5 years (2.9–8). The 2 subgroups of children did not differ in age or gender.

In the group of children sensitized to HDM, we observed a severe phenotype of AD in 24 (49.0%) children, and in the group of children without sensitization – in 6 (11.5%) children. This difference was statistically significant (OR = 7.360 (2.658–2.382)). Early age of onset of AD (up to 18 months) occurred in 45 (91.8%) children who had sIgE to HDM, and in 43 (82.7%) children insensitive to HDM. We did not find an effect of early phenotype on sensitization to HDM (OR = 2.35 (0.675–8.217)). The presence of a paternal history of atopy also did not affect the development of sensitization: paternal history was present in 21 (42.9%) sensitized and 16 (30.8%) non-sensitized children (OR = 1.688 (0.746–3.819)).

Among 49 children sensitized to dust mites, 13 (26.5%) children had concomitant asthma and 22 (44.9%) children had AR, among whom 11 (22.4%) had combined allergic respiratory disease. Among 51 children without sIgE to HDM, 6 (11.5%) patients had concomitant asthma, 12 (23.1%) - AR, and 2 (3.8%) - both allergic diseases. The presence of sIgE to HDM in patients with AD was significantly associated with concomitant AR (OR = 2.593 (1.096–6.133)) and in the group of children with both concomitant allergic diseases (OR = 7.432 (1.553–35.564)).

This is the first study of the rs7309123 polymorphism in the Ukrainian population. Our study revealed the difference in the rs7309123 genotype distribution in the Dectin-1 gene in patients depending on the presence of sIgE to HDM. In the subgroup of children with AD who were sensitized to HDM, 12 (15.0%) children had the CC genotype, 20 (23.0%) were heterozygous, and 16 (10.0%) had the GG variant. Among the non-sensitized children, 14 (12.0%) patients had the CC variant, 32 (23.0%) were heterozygous, 6 (17.0%) had the GG genotype (OR = 3.111 (0.924–10.480)) for CC and GG OR = 4.267 (1.432–12.716) for CG and GG). There was a statistically significant difference in the distribution of genotypes in patients with elevated serum IgE to HDM and among children with normal levels (χ^2 = 7.320; p < 0.05) (Figure 1). Thus, percutaneous sensitization to HDM depends on this genetic variant of the rs7309123 polymorphism in the Dectin-1 gene (Table 1).

DISCUSSION

This study found that 48.5% of children with AD had sensitization to HDM. Children, sensitive to HDM, and children not sensitized did not differ in age and sex, the pres-

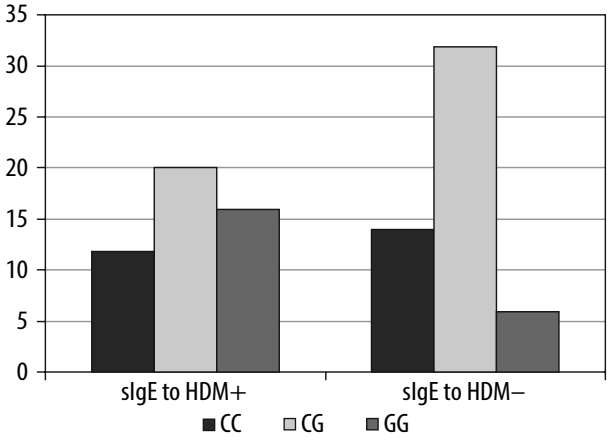

FIGURE 1. Genotypic distribution of Dectin-1 rs7309123, comparison between HDM-sensitized and non-sensitized children

*p < 0.05.

ence of atopy in the history of parents, and the early onset of the disease. Children who were sensitized to HDM had significantly more severe AD (OR = 7.360 (2.658–2.382)). Patients with AD have impaired epidermal barrier function, which provides easy access to the epidermis for proteins, microbes, and other irritants. These particles may interact with local immune cells and initiate immediate and delayed-type hypersensitivity reactions that are common in patients with AD. Sensitivity to HDM in patients with AD has been previously studied using various methods (skin tests, specific IgE to extracts of allergens or molecular components). As a result of the heterogeneity of the studied populations and the applied methods, the research results differ significantly [10, 11]. Yazıcı *et al.* [12] studied sensitization in a group of children with AD, AR, asthma, and urticaria using skin tests. 32.7% were sensitive to Der f and 30.4% to Der p. In the Ibrahim HM study [13], 44% (n = 22) of patients were positive for serum IgE to HDM extract. González-Pérez *et al.* [14] found in 80 patients with AD that the major allergens (Der p 23, Der p 2, and Der p 1) were present in more than 86% of all subjects, regardless of severity. Wisniewski *et al.* [15] showed that the prevalence of HDM sensitization in patients with AD depends significantly on age: it increases by up to 10% among children under 2 years old, to more than 50% for children after 5 years old. Also in this study, children with eczema who were sensitive to HDM had respiratory allergic diseases. In a study by Aranda CS, mite sensitization was the most frequent in 470 children with allergy, with a predominance of Der p 1 and Der p 2 sensitization (78.3% and 79.4%, respectively) [16].

Epidermal sensitization to dust mites may be an important prerequisite for the progression of eczema into respiratory allergy. It was found that in the group of children sensitive to HDM, asthma and AR were significantly more common. The presence of sIgE to HDM in patients with AD was significantly associated with concomitant AR (OR = 2.593 (1.096–6.133)) and in the group of children with both concomitant AR and asthma (OR = 7.432 (1.553–35.564)). It has been recognized that sensitization to common inhaled allergens develops in most patients with AD, often during AD. In addition, the early onset and severity of AD are related to the degree of sensitization to air allergens and the risk of developing asthma. Although studies suggest that atopic skin may provide signals that

TABLE 1. Anamnestic, clinical, and genotypic data of patients with atopic dermatitis

Parameters		Sensitized to HDM n = 49 (48.5%)	Non-sensitized to HDM n = 52 (51.5%)	OR
Severe AD/Mild + moderate AD		24 (49.0)/25 (51.0)	6 (11.5)/46 (88.5)	7.360 (2.658–2.382)
Early age of onset (up to 18 months), n (%)		45 (91.8)	43 (82.7)	2.35 (0.675–8.217)
Parental history of atopy, n (%)		21 (42.9)	16 (30.8)	1.688 (0.746–3.819)
Concomitant allergic diseases, n (%)	Asthma	13 (26.5)	6 (11.5)	2.769 (0.958–8.000)
	AR	22 (44.9)	12 (23.1)	2.716 (1.154–6.394)**
	Asthma + AR	11 (22.4)	2 (3.8)	7.237 (1.514–34.598)**
Genotype, n (%)	CC	12 (11.9)	14 (13.9)	3.111 (0.924–10.480)
	CG	20 (19.8)	32 (31.7)	4.267 (1.432–12.716)**
	GG	16 (15.8)	6 (5.9)	1.00
Dominant model	CC	12 (11.9)	14 (13.9)	1,00
	CG + GG	36 (33.3)	38 (36.4)	1.105 (0.451–2.707)
Recessive model	CC + CG	32 (31.7)	46 (93.9)	1.00
	GG	16 (15.8)	6 (5.9)	3.833 (1.353–10.857)**

**Statistically significant result at $p < 0.05$. HDM – house dust mite, AR – allergic rhinitis.

may contribute to the sensitization to inhaled allergens, thereby triggering an "atopic march", the mechanism of this phenomenon remains unclear.

C-type lectin receptors are involved in recognition and induction of adaptive immunity to pathogens, and their role in the recognition of allergens and the induction of allergic inflammation has been discussed in advance [17, 18]. Kanemaru found that a mucin-like molecule in HDM is a ligand for mouse Clec10a; thus, Clec10a in mice plays an important role in skin inflammation associated with HDM-induced dermatitis [19]. Dectin-1 seems to play an important role in the induction of HDM-induced allergic airway inflammation [8, 9]. The study of HDM-induced allergic airway lung inflammation transcriptome analysis of BMDCs revealed that Dectin-1 signalling enhances the expression of CCL3 and CCL4, which induce the recruitment of monocytes, NK cells, and memory T cells [8]. Expression of CCL1, CCL3, and CCL4 mRNA is elevated in patients with acute AD [20]; therefore, this receptor may be an important mediator of skin inflammation. Our study demonstrated that a genetic variant in Dectin-1 gene may affect its function, presumably altering dust mite antigen recognition, which contributes to the allergic inflammation. The data of this study showed statistically significant differences in the distribution of genotypes in patients with elevated serum IgE to HDM and among non-sensitized children (χ^2 = 7.320; $p < 0.05$). The genetic variant of the GG polymorphism rs7309123 of the Dectin-1 gene promotes percutaneous sensitization to HDM in patients with AD.

CONCLUSIONS

This study showed that the susceptibility to dust mites among children with AD is 48.5%. The presence of sensitization to HDM in patients with AD significantly determines the phenotype of the disease, which is determined by its severity and the development of concomitant respiratory allergic diseases. Dectin-1 is involved in the development of HDM-induced allergic inflammation in skin because polymorphism rs7309123 of the Dectin-1 gene may affect percutaneous sensitization to HDM in patients with AD. The inhibition of signalling pathways of Dectin-1 may be an efficient therapeutic strategy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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