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Corneometric features in atopic dermatitis under the conditions of filaggrin dysmorphism in children

O. V. Reshetylo

Odessa National Medical University

Abstract

The objective: to study the morpho-functional state of the skin in atopic dermatitis in children, taking into account the polymorphism of filaggrin protein genes. **Materials and methods.** 111 children with atopic dermatitis (AD) aged 3 - 11 years old were examined. In addition to general-clinical and allergic examination, a molecular-genetic analysis was conducted to detect polymorphism in the filaggrin gene (FLG). The hydration of the skin was measured by corneometry. Statistical processing of the material was performed using Statistica software 6. **Results.** It has been established that in 51 AD children (45.9% ± 6.98) polymorphism of FLG took place. Mutation of R501X occurred in 40 children (78.4 ± 5.76%), polymorphism of 2282del4 had 4 patients (7.8 ± 3.76%), combined variant of R501X and 2282del4 took place in 7 (13.7 ± 4.81%) patients. The influence of FLG structural changes on the morpho-functional state of the skin was revealed. It was reflected by a significant decrease in the moisture content of the epidermis ($p < 0.05$). In all main group patients the mean corneomy index was less than 45 points and in 17 children (33.3 ± 6.6%) ≤ 30 points, which corresponds to the status of "very dry skin"; in the control group only 18 children (30% ± 5.9) had skin moisture content less than 45 points; there were none patients with corneometry indices ≤30 points in the control group. The clinical course of AD in the presence of FLG polymorphism is characterized by an early debut (up to 3 months; $p < 0.001$) and more severe course of the disease ($p < 0.001$); predominaning sensitization to fungal and

domestic allergens (p <0.05). **Conclusions.** Polymorphism in FLG in AD children was found in 45.9% of the patients under examination. R 501X mutation was found in 78.4% of children, 2282del4 polymorphism in 7.8% of patients, its combined version (R501X and 2282del4) took place in 13.7% of patients. FLG polymorphism leads to a significant reduction in the skin's moisture in AD children, triggers its early debut and more severe course, promotes sensitization to domestic and fungal allergens.

Key words: atopic dermatitis, children, filaggrin, corneometry.

Introduction. Atopic dermatitis (AD) is a widespread inflammatory cutaneous disease which increasingly debuted in the first year of life [1]. In recent decades there has been a continuous growth in AD morbidity rate in developed countries. For instance, in 2016, the incidence of AD in children in the United States ran high by 17.2%, in Europe by 15.6% [2]. In Russia AD morbidity rate during the same period ranged from 6.2 to 15.5% depending on the region [3].

Foreign authors note that AD affects up to 20% of children and up to 3% of adults (S. Nutten, 2015; E. R. Notaro, R. Sidbury, 2015).

In 2012 an International Study on Asthma and Allergies in Childhood (ISAAC) was completed. There were about 2 million children from 100 countries under examination. It the study high prevalence of AD in children was noted in European countries. Thus, in Austria its prevalence was 9.7 - 20%, in Latvia – 5.4-6.5%, in Poland – 13.2%; the prevalence of AD among 6 - 7 y. o. children ranged from 0.9% in India to 22.5% in Ecuador. For 13 - 14 y. o. children the incidence of AD varied from 0.2% in China to 24% in Colombia with higher rates in Africa and Latin America.

Although today the pathophysiological processes occurring in the AD body are quite widely studied, the issue of the significance of the genetic basis and their role in the development and features of the disease remain undisclosed. At the present stage, the role of genetically determined immunological disturbances, which create an immunopathophysiological platform for atopy realization and the significance of hypersensitivity to allergens and to non-specific stimuli, colonization by pathogenic microorganisms (*Staphylococcus aureus, Malassezia furfur)*, imbalance of the nervous system with increased production of inflammatory mediators has been proved [3].

However, these pathogenetic aspects are not enough to fully explain the peculiarities of inflammatory processes in the skin that occur in AD. Probably, these are the molecular genetic disorders that lead to the failure of the epidermis to provide a barrier function, are

fundamental for the transcutaneous penetration of allergens and the loss of moisture and form the conditions for chronic inflammation of the skin.

Today FLG, an epidermal protein, is important, as it creates a protective barrier, thereby preventing the loss of moisture and the penetration of allergens and microorganisms through the keratinous skin layer [4].

FLG is a product of proteolytically modified profilaggrin, a precursor, containing 324 multiple filaggrin units, and is present in keratogialin granules. This is an intermediate filament-associated protein that combines keratin fibers in epidermis.

It is believed that polymorphism in the filaggrin gene is the most important genetic factor in AD development, and hence the entire atopic chain [5]. There are published data on the influence of filaggrin polymorphism on the formation of bronchial asthma phenotype in AD patients [6].

Filaggrin is a key protein involved in the ultimate differentiation of keratinocytes and the formation of a cutaneous barrier. Epidermal proteins genetic defects, such as filaggrin, are important in the development of AD.

At present more than 40 mutations with the creation of a stop codon specific for different populations and races are established [6]. The most thoroughly rummaged mutations of filarrgin gene are 2282del4 and R501X, located in the third exon. There are data on the major mutations in FLG in Europeans: R501X, 2282del4, S3247X, 3702delG, R2447X [8]. According to Weidinger et al., two FLG's mutations (R501X and 2282del4) are most significantly associated with the risk of AD development [9], besides, their role in the development of other allergic diseases: allergic rhinitis, adult bronchial asthma [10] has been proven. However, the prevalence and diversity of FLG polymorphism in children was not investigated.

Materials and methods

111 children aged 3 - 11 years old participated in the research and gave PIC. Diagnosis of AD was exposed on the basis of complaints, anamnestic data, clinical examination, laboratory data with the definition of general and specific Ig E. The severity of AD was assessed on the SCORAD scale. Each patient's buccal epithelium scraping from mucous membrane of the oral cavity was taken for molecular-genetic analysis. The epithelium was collected in an Eppendorf tube with a sterile physiological solution. All biomaterials received were transported to the laboratory in special thermocontainers at a temperature of 4ºC. Isolation and purification of DNA from buccal cells was performed by the method of

Dellaport, 1983. (Dellaporta S.L., Wood J., Hicks J. B., Plant DNA MiniPreparation: Version2 // PlantMol.Biol.Rep 1983.V.I.P. 19-21.)

To evaluate the morpho-functional condition of the skin craniometry method was used which allows directly determine the degree of epidermis hydration. The basis of the corneometer work is the principle of capacitance determining (changes of cutaneous dielectric properties depending on the amount of water content in the keratoconus epithelium). The skin is a dielectric medium where, accordingly, fluctuations of the dielectric constant occur due to changes of moisture content in the surface layers, which entails changes in the capacitive characteristics of the organ under study. Corneometer has significant advantages: the depth of penetration of electric waves is reliably small, therefore, the moisture content is measured only in the corneum layer, the effect of the lower tissues' capacity on the result of measurement is impossible. Due to the short-term measurement its distortion through occlusion is excluded. The device has small sizes (looks like the ballpoint pen), it is portable, easy to operate. The sensor's shape and size allows the measurements to be made on any parts of the body. Corneometry is a semi-quantitative method, the result of measurement is stated in conditional units (scores). Portable Cornimeter Monaderm 98000 MONACO Scale has a range from 0 to 99 units. The measurement result of less than 30 scores denotes to a very dry skin, from 30 to 60 - to a dry skin, over 60 to a wetted of varying degrees skin.

In order to minimize the influence on environmental temperature and humidity on the parameters of corneometry, a patient was at least 15 minutes in a room at a temperature of 22-23^{o}C. To exclude the effect of moisturizers, which AD patients often use, they were instructed not to apply emollients on the skin for a day before corneometry.

Statistical processing of the material obtained was carried out with the use of Statistica 6 software. To compare clinical, laboratory and instrumental data of AD course in different groups, the significance of the indicators difference was evaluated using the non-parametric Pearson $\chi 2$ criterion with the correction for continuity. At an expected frequency of 5 to 9, Yates' correction was used; at the expected frequency less than 5, Fisher's exact criterion was used. Differences were considered statistically significant at $p < 0.05$.

Results. As a result of a genetic survey made, it was found that in 51 AD children (45.9% ± 6.98) there was FLG polymorphism. R501X mutation occurred in 40 children (78.4 ± 5.76%), polymorphism of 2282del4 was in 4 patients (7.8 ± 3.76%) and combined R501X and 2282del4 variant was revealed in 7 (13.7 ± 4.81%) patients.

*Determination of FLG polymorphism effect on the course of AD was performed by comparing the clinical and laboratory characteristics of 51 children with existing

polymorphism R501X and 2282del4 (the main group) and 60 patients without polymorphism (control group).

Significant age differences in the comparison groups have not been established, however, the average age in the main group was slightly higher (7.2 ± 2.1), than in the control group - 6.5 ± 2.1 p. According to the gender characteristics the groups under examination did not differ significantly. There were 26 boys and 25 girls in the main group and 28 boys and 32 girls in the control one. In the main group the mild course of AD was established in 7 children (13.7 ± 4.81%), moderate in 16 (31.3 ± 6.49%), severe in 28 (54.9 ± 6.97%) patients. In the control group the results were significantly different and a mild course was observed in 20 (33.3 ± 6.08%) patients, moderate in 29 (48.3 ± 6.45%), and severe in 11 (18.3 ± 4.99%) patients. In the control group there was a tendency to decrease the number of patients with severe AD due to an increase in the percentage of children with mild course of illness.

Features of the clinical course of AD were characterized by polyvalent sensitization. In the control group, for the most part, the debut of the disease was provoked by food allergens. According to the definition of specific Ig E in serum, the sensitization was more often determined to beef (28.3 ± 5.8%), rice (25 ± 5.6%), cow's milk (23.3 ± 5.5%), buckwheat (23.3 ± 5.5%), chicken eggs (16.7 ± 4.8%), wheat (18.3 ± 4.9%), oatmeal (15 ± 4.6%) and potatoes (15 ± 4.6%). In the main group, domestic and fungal allergens were more important: sensitization to *D. farina* and *D. pteronisin* was observed in 72.5 ± 6.25% of children. There was allergization to fungal allergens in 90.2 ± 4.2% of children, to penicil – in 52/9 ± 7.0%, to *asperg Niger* – in 43.1 ± 6.9% and to *Alternar altern* – in 58.5 ± 6.9% patients.

Analysis of the hereditary history in the main group allowed to detect hereditary burden in 60.8 ± 6.84% of patients, in the control it was determined in 50 ± 6.45% of children. AD in the relatives of the first line in the main group was found in 72.6 ± 6.25% of patients, so AD was recorded at least in one of the parents in 62.7 ± 6.77% of cases, and bronchial asthma in 41.2 ± 6.89% of cases.

An early manifestation of AD symptoms was established in the main group, so in 42 children (82.4 ± 5.33%) the first signs of AD appeared for up to 3 months, and in the contrast, in the control group the y took place only in 17 (28.3 ± 5.82 %) children. In the most control group children the appearance of AD symptoms occurred in the period from 3 to 6 months (31 children, 51.6 ± 6.45%), older than 6 months tere were 12 (20 ± 5.16%) patients. The data obtained confirm that the age-dependent peculiarities of AD clinical course are associated

with the morphological and functional features of the skin characteristic typical for a certain age.

A child's skin has certain anatomical and physiological features that explain its vulnerability to trigger effects. Less thickness of epidermis, nondense structure, high water content in corneocytes and their rarefied location, thin and loose horny layer contributes to rapid loss of moisture in early and younger age children. The formation of elastic fibers begins after 3 months of age, the water-lipid layer, which has antibacterial properties and should protect against negative environmental effects and excessive loss of moisture is underdeveloped in children and contains thrice less lipids. Sebaceous glands are quite active in the first year of life, then their activity decreases until puberty. Only after 6 – 7 years of age the architectonics of a child's skin approaches the structure of an adult's skin [11]. So, the early onset of AD in the children under examination can be explained by morpho-functional epidermis changes associated with polymorphism in filaggrin gene.

While investigating, it was found that in the main group, the average value of the SCORAD index was 52.8 ± 7.5 points, in the control group 33.0 ± 4.0; $p < 0.05$; the area of defeat was 52.1 ± 13.2%. In the control group SCORAD index was 19.5 ± 10.2%; $p < 0.001$. Respectively, the severity of objective clinical signs in the main group was 10.2 ± 1.7 points, in the control one - 7.2 ± 1.3 points, $p > 0.05$; intensity of itching in the main group was 3.5 ± 0.5 points, in the control – 2.3 ± 1.1 points, $p > 0.05$; sleep disturbance in the main group constituted 3.2 ± 0.6 points, and in the control one 1.8 ± 0.6 points, $p > 0.05$.

To study the morpho-functional characteristics of the skin, corneometry was carried out, the results of which are given in Table 1

Table 1

The results of corneometry in the the groups of children under examination

	AD with FLG polymorthism	AD without polymorphism	Reference value	p		
	1	2	3	1-2	1-3	2-3
CM_1	25.0±4.7	49.1±4.7	76.6±10.8	<0.01	<0.001	<0.01
CM_2	25.45±3.8	49.1±4.8	75.0±12.1	<0.01	<0.001	<0.01
CM_3	28.4±5.5	47.2±4.6	77.7±11.8	<0.05	<0.001	<0.01
CM_4	29.8±5.7	46.9±6.1	75.3±8.9	>0.05	<0.001	<0.01
CM_{max}	41.0±5.9	62.0±5.2	97.0±11.7	<0.05	>0.05	<0.001
CM_{min}	17±5.9	39.0±5.2	53.0±11.7	<0.01	<0.001	>0.05
CM_{avr}	27.2±5.5	48.3±5.7	75.0±11.7	<0.05	<0.001	<0.01

While analyzing the results of corneometry, it was found that in the main group children skin humidity is significantly lower in comparison with the control group.

The face and upper limbs are the driest zones of the body in children with polymorphism R501X Aa, 2282 del4 which may be due to increased sensitivity of the skin to the influence of the environment, since these anatomical zones are usually always open and often come in contact with detergents, water, undergo significant temperature fluctuations, and so on. Apparently, in AD children under conditions of polymorphism in filarrgin philagrin gene a more active transepidemal loss of moisture than at AD without disturbing filarrgin's synthesis. The statistically significant increase in the dryness of the epidermis in children of the main group is clearly evident when comparing the minimum corneometry indices. It is characteristic that in all main group patients mean corneomy index was less than 45 points, and in 17 children (33.3 ± 6.6%) even ≤30 points, which corresponds to the status of "very dry skin", at the same time in the control group only 18 children (30% ± 5.9) the skin's moisture was less than 45 points, while the patients with corneometry ≤30 points were not observed at all in the control group.

Conclusions:

1. 45,9% ± 6,98 AD children have polymorphism in the protein of filarrgin gene. Mutation of R501H was found in 78.4 ± 5.76%, polymorphism of 2282del4 was in 7.8 ± 3.76% of the patients, the combined variant of R501X and 2282del4 was found in 13.7 ± 4.81% of patients.

2. As a result of AD course study, the effect on FLG structural changes on the morpho-functional state of the skin has been proved. This is manifested by a significant decrease in the moisture content of the epidermis; corneometry indices in children with AD associated with FLG polymorphism are significantly lower than in patients without polymorphism so the average index of corneometry in the main group was 27.2 ± 5.9, and in the control one - 48.3 ± 5.7; $p < 0.05$. Minimum corneometric index in the main group was 17 ± 5.9, and in the control - 39.0 ± 5.2, $p < 0.01$; the maximum corneometry index in the main group was 41.0 ± 5.9, in the control - 62.0 ± 5.2, $p < 0.05$. The clinical course was characterized by an early debut (up to 3 months), and a more severe course of the disease, $p < 0.001$. This was accompanied by sensitization to fungal and domestic allergens, $p < 0.05$.

References:

1. Dinulos JG, Trickett A, Crudele C. New science and treatment paradigms for atopic dermatitis. Curr Opin Pediatr. 2018;30(1): 161-168. doi: 10.1097/Mop.0000000000000560.

2. Egawa G, Kabashima K. Barrier dysfunction in the skin allergy. Allergol Int. 2018;67(1):3-11. doi: 10.1016/j.alit.2017.10.002.

3. Petrova IV, Omarov NN, Sargsyan MS, and others. Supportive pharmacotherapy of atopic dermatitis // Reviews of clinical pharmacology and drug therapy. 2018. T. 16. No. 1. P. 60-63. doi: 10.17816 / RCF16160-63) [Russian].

4. Akan A, Azkur D, Ginis T, et al. Vitamin D level in children is correlated with severity of atopic dermatitis but only in patients with allergic sensitizations. Pediatric Dermatology. 2013;30(3):359-63, 2013. doi: 10.1111/pde.12058

5 Dyatytkovskii VO Atopic March in Pediatrics: The Genotype-Associated Mechanisms // Child Health.- 2017.- Vol. 12.- No. 4.- P. 498-502 [Ukrainian].

6. A new set of reagents of the Realbest-Genetic series in assessment of prevalence of mutations of Filaggrin gene among residents of Novosibirsk and children with atopic dermatitis / E. G. Komova [et al.] // News of «Vector-Best». — 2014. — No 4.

7. The functional significance of single nucleotide polymorphism (rs11204981) in the filaggrin gene (FLG) for the treatment of bronchial asthma in children with atopic dermatitis ./ Volosovets O. P., Dosenko V. S., Krivopustov S. P., Pavlik O. V., et al. // Child health.- 2015.- №3. – P. 5- 11 [Russian]

8. Zuyeva M. I. Odnonukleotidny and insertional nene^oHHtrn a polymorphism of a gene of FLG of the person at dermal diseases / M. I. Zuyeva, D. O. Parfyonov, L. A. Atramentov//Factors of experimental evolution of organisms. — 2013. — T. 13. — P. 303-306.

9. Papa V. Analysis of mutations in the filagargin gene in patients with atopic dermatitis, genetically obstructed: Zb sciences works of P.L. Shupyk NMAPO employers .- 2013.- N 22 (3). -P.101-106 [Ukrainian]

10. Seon-Young Kim, Sung Wan Yang et al. Association between P478S polymorphism of the filaggrin gene & atopic dermatitis // Indian. J. Med. Res. — 2013. — 138(6). — 922-927.

11. Skin features in young children and approaches to preserving its physiological state / L.V. Kvashnina, I.N. Matvienko // Modern Pediatrics.- 2017.- N 2 (82).- P. 502 [Russian].