THE ROLE OF GENETIC POLYMORPHISM OF THE FILAGGRIN PROTEIN WITH ATOPIC MARCH PROGRESSION IN CHILDREN

Abstract. The aim of the study. To determine the role of genetic polymorphism in the filaggrin gene R501XAA and 2282de4AA at atopic march progression in children. Materials and methods. 111 children aged 3 to 12 years with atopic dermatitis were selected and examined. As a result of genetic testing, it was found that 51 children with atopic dermatitis had polymorphism in the filaggrin gene. These patients were included in the main group. Another 60 children without polymorphism were in the control group. The filaggrin gene polymorphism was determined by
examining the buccal epithelium by Dellaporta method. Sensitization to allergens was established on the basis of the specific IgE level. The impact of the disease on the quality of life of children was performed using the CDLQI questionnaire (Children’s Dermatology Life Quality Index). Results. In the course of molecular genetics research, R501X mutation was detected in 40 (78.4 ± 5.76)% children, 2282del4 polymorphism – in 4 (7.8 ± 3.76)% patients, and their combined variant R501X + 2282del4 – in 7 (13%), (7 ± 4.81)% patients. When determining the effect of filaggrin polymorphism on the clinical course of atopic dermatitis, the presence of the associative relationship was established with the following indicators: the early onset of the disease – χ² = 33.2, mostly severe course – χ² = 16.2, severe skin dryness – χ² = 22.6, predominant sensitization to fungi – χ² = 10.6 and house dust mites – χ² = 12.2, violation of the skin microbiome – χ² = 7.8. Conclusions. Early manifestation of atopic dermatitis in children is associated with the filaggrin protein gene polymorphism (82.4 ± 5.33%), which determines the risk of progression of the atopic march and the development of bronchial asthma in (38.0 ± 6.8)% of children. 

**Keywords:** children, atopic dermatitis, bronchial asthma, filaggrin.

**Introduction.** The prevalence of allergic diseases is steadily increasing around the world, and there is a tendency to increasing complexity and severity of allergic processes in childhood.

Recently, significant progress has been made in understanding the pathophysiology of allergy, through the use of molecular diagnostics, immunophenotyping, tissue engineering and the study of epidermal nanostructures. These studies have shown that the basis of allergic processes is complex heterogeneous pathophysiological mechanisms, covering various phenotypes and endotypes of pathology, which determine the development and subsequent prognosis of the disease.

A special place in the structure of allergic diseases is occupied by atopic dermatitis (AD), which is one of the most common inflammatory skin diseases and occurs in children with a frequency of 15–20%. Beginning often at an early age, the disease has not only pronounced clinical manifestations, but also significantly affects the quality of life of the child.

Although the etiopathogenetic mechanisms that characterize the development of allergic pathology have been studied in great detail, the importance of non-immune mechanisms in AM pathophysiology and development remains unclear.
Currently, the role of genetically determined disorders, which create an immunopathophysiological platform for the realization of atopy, hypersensitivity to allergens and non-specific stimuli, hyperproduction of inflammatory mediators in allergic conditions has been proved [1]. However, these pathogenetic aspects are not enough to fully explain the features of inflammatory processes in the skin that occur during AD and the atopic march (AM) progression. Probably, the disturbances at the molecular level underlie the inability of the epidermis to provide a barrier function and prevent transcutaneous penetration of allergens, which ultimately creates the conditions for the formation of chronic inflammation in the skin and bronchi.

Today the data on the participation of 26 genes in the development of atopic inflammation have been released. The most of them relate to a certain allergic condition [2]. According to GWAS guidelines (genome-wide association studies), genes associated with allergic diseases are divided into two groups: skin barrier genes and immune response genes. In some patients with allergic pathology, both directions intersect, so initiating a chain of inflammation that will be responsible for the development of certain clinical nosoforms – AD, bronchial asthma (BA), allergic rhinitis (AR) – or their combination [3–5].

In the group of skin barrier genes, a special place is occupied by the filaggrin protein (filament aggregating protein – FLG), which is responsible for the regulation of epidermal homeostasis [6]. Precisely because of the filaggrin protein, the protein-lipid structure of the stratum corneum is formed, the differentiation of keratinocytes takes place, creating a barrier that prevents moisture loss and penetration of allergens and microorganisms [7]. FLG is a product of proteolytically altered profilagrin, a precursor that contains 324 multiple filaggrin units and is found in keratohyalin granules. It is proved that the intermediate filament-associated protein combines keratin fibers in the epidermis. Under conditions of the allergic process, keratinocytes of the broken barrier secrete immune adjuvants that activate the maturation of Langerhans cells or dendritic cells, as well as affect their ability to direct the polarization of naive Th-lymphocytes and, accordingly, affect the Th-response nature. The key structural proteins of the upper layer of the epidermis
involved in keratinization are encoded on chromosome 1q21, the gene encoding filaggrin is found in this locus [8, 9].

So, polymorphism in FLG gene is the most important genetic factor in AD development, and probably atopic march in general [10] due to the proved role of mutations in FLG gene at the level of 1q21, which causes violation of the filaggrin synthesis, inducing the development of not only AD but also BA [1, 11]. The search for the role of the filaggrin protein defects in the development of BA in children is under way [8].

Currently, the active study of FLG gene polymorphism is under way and more than 40 mutations specific to different populations and races have been described today [12, 13]. The most common mutations were in the third exon – 2282del4 and R501X. In European regions, the most common variants are: R501X, 2282del4, S3247X, 3702delG, R2447X, and in Asian countries – 3321delA (East Asia), and Q2417X (Taiwan and China). Interestingly, in Russia the polymorphism R501X, 2282del4, S3247X, R2447X occurs in patients with AD with different frequency, but the carriers of the mutation 3702delG are not detected at all [14, 15]. These data clearly demonstrate the difference in FLG population genetics between Europe and Asia [16].

By prevalence, mutations in FLG gene on chromosome 1q21 occur in 50–60% of Europeans suffering from AD. At the same time, according to national studies, mutations in R501X and 2282del4 in FLG gene are observed in only 20.6% of patients with AD, which obviously does not reflect the real situation, but indicates insufficient involvement of molecular genetic analysis in AD diagnosis [17].

The question of the role of FLG polymorphism variants in the development of the atopic march remains open. According to existing data, FLG mutations (R501X and 2282del4) are responsible for the coexistence of AD, allergic rhinitis, bronchial asthma, single-nucleotide polymorphism SNPs – AD and bronchial asthma, polymorphism rs11204981 – bronchial asthma [18–20].

Focusing on the problem in the pediatric population has shown that there is a lack of studies of the FLG polymorphism role in allergic pathology of children. The few published data report that in children with AD of eastern countries, FLG
2282del4 mutation is registered with a frequency of 12.62%. In the pediatric population of Ukrainians, the FLG rs11204981 polymorphism takes place in children with bronchial asthma, with 5% of patients having the minor allele, 27% – the heterozygous allele, and 67% – the major allele [18, 21].

The aim of the study. To determine the role of genetic polymorphism in the filaggrin gene R501XAA and 2282de4AA in atopic march progression in children.

Materials and methods

As a result of screening, 111 children aged 3–12 years old with atopic dermatitis were selected and examined to solve the set tasks.

The diagnosis of atopic dermatitis is established according to current national and international guidelines. To determine the polymorphism of FLG gene R501XAA and 2282de4AA with AD in children, the buccal epithelium was examined. Isolation and purification of DNA from buccal cells was performed by S.L. Dellaporta method (1983). To assess the quality of life of children the questionnaires based on CDLQI (Children's Dermatology Life Quality Index) was used, the questionnaire CDLQI/mod 1 – for children from 3 to 7 years, the questionnaire CDLQI – for children from 7 to 12 years. Allergy tests were performed in vitro to determine the level of specific IgE.

Research results and their discussion

As a result of genetic testing, it was found that 51 ((45.9 ± 6.98)%) children with AD had polymorphism in the FLG. These patients were included in the main group, another 60 children without polymorphism were in the control group. In the course of molecular genetic research, R501X mutation was detected in 40 ((78.4 ± 5.76)%) children, 2282del4 polymorphism – in 4 ((7.8 ± 3.76)%) patients, and their combined variant R501X + 2282del4 – in 7 ((13.7 ± 4.81)%) patients. The distribution of allelic variants of FLG polymorphism taking into account the gender of patients is given in Fig. 1

The results in Fig. 1 demonstrate that the variants of FLG gene polymorphism were distributed almost equally in girls and boys: R 501 X Aa mutation predominated in both groups. A slight increase in mutations 2282 del 4del was observed in boys, while a combined variant was presented with the same frequency,
regardless of gender.

![Gender distribution of allelic variants of the filaggrin in patients](image)

Determination of the influence of polymorphism in FLG gene on the AD course was performed by comparing clinical and laboratory characteristics in the main and control groups. During analysis of significant age differences in the comparison groups were not found. However, the average age in the main group was slightly higher (7.2 ± 2.1) vs. (6.5 ± 2.1) years, p>0.05). The groups did not differ significantly by gender: in the main group there were 26 (51%) boys and 25 (49%) girls, in the control – 28 (46.48%) boys and 32 (53.12%) girls. When determining the effect of FLG polymorphism on the severity of the disease it was found that in the main group, mild AD was found in 7 (13.7 ± 4.81)% children, moderate – in 16 (31.3 ± 6.49)% severe – in 28 (54.9 ± 6.97)% patients. In the control group, the results differed significantly: the mild course was observed in 20 (33.3 ± 6.08)% patients, moderate – in 29 (48.3 ± 6.45)% and severe – only in 11 (18.3 ± 4.99 )% patients. That is, in the control group there was a tendency to reduction of patients with severe AD due to an increase in the percentage of children with a mild disease course.

The analysis of hereditary anamnesis in the main group of the study revealed hereditary burden in (60.8 ± 6.84)% of patients, and in the control group in (50 ± 6.45)% of children (p>0.05). The diagnosed AD in first degree relatives at the main group is documented in (72.6 ± 6.25)% cases. The presence of AD in at least one of
the parents was registered in \((62.7 \pm 6.77)\%\), and the presence of BA – in \((41.2 \pm 6.89)\%\) of cases.

Features of the allergic response with AD in the examined children were characterized by polyvalent sensitization. In the control group, the onset of the disease was mostly provoked by food allergens and according to the determination of specific IgE in the blood serum was more often determined sensitization to beef – \((28.3 \pm 5.8)\%\), rice – \((25 \pm 5.6)\%\), cow's milk – \((23.3 \pm 5.5)\%\), buckwheat – \((23.3 \pm 5.5)\%\), chicken egg – \((16.7 \pm 4.8)\%\), wheat – \((18.3 \pm 4.9)\%\), oatmeal – \((15.0 \pm 4.6)\%\) and potatoes – \((15.0 \pm 4.6)\%\). In the main group, household and fungal allergens became more important: sensitization to \(D. farina\) and \(D. pteronyssinus\) was observed in \((72.5 \pm 6.25)\%\) of children, to fungal allergens – in \((90.2 \pm 4.2)\%\), in particular to \(Renicillium\) – \((52.9 \pm 7.0)\%\), \(Aspergillus niger\) – \((43.1 \pm 6.9)\%\), \(Alternarnaria tenius\) – \((58.5 \pm 6.9)\%\).

It is obvious that antigenic influence through damaged skin is sufficient to cause systemic (Th2) allergic inflammation in various systems, and can help to explain the development of AM in which AD is the earliest atopic manifestation. In addition, it emphasizes the importance of skin sensitization by allergens, even if allergic inflammation occurs in distant organs.

The peculiarity of the clinical course is early manifestation of AD symptoms in the main group. So, 42 \((82.4 \pm 5.33)\%\) children had the first signs of AD appeared before 3 months, in contrast, the control group patients had early signs only in 17 \((28.3 \pm 5.82)\%\) cases, \(p<0.01\). The onset of AD symptoms in the majority of control group children occurred at the period from 3 to 6 months \((31 \,(51.6 \pm 6.45)\%)\) and from 6 to 18 months \((12 \,(20.0 \pm 5.16)\%)\) patients. The obtained data confirm that the age-related features of the clinical course of AD are associated with morphofunctional peculiarities of the skin typical for a certain age. Anatomical and physiological features of the skin of infants and young children, such as smaller thickness of the epidermis, its looser structure, significant water content in corneocytes and their sparse location, thin and loose stratum corneum, immaturity of elastic fibers and water-lipid layer, combined with molecular genetic preconditions and violation of structural and functional properties of basic proteins
of the epidermis, especially filaggrin, explain its increased sensitivity to numerous trigger factors.

The study of the AD course peculiarities, under conditions of FLG genetic polymorphism, showed the association with the severe course of the disease in (54.9 ± 6.97)% of children. While analyzing the disease course depending on the variant of FLG polymorphism, the worsening of AD course in the presence of R501X Aa 2282del4 or R501XAA 2282del4 del in (50.0 ± 4.9)% was determined, the severe course was determined in more than 70% patients in case of these mutations combination. The course of moderate severity was diagnosed at R501HAA 2282del4 del in (25.0 ± 4.3)% of children, at R501Xa 2282del4 – in (32.5 ± 4.8)% of children. The AD course of mild severity was not registered with combination R501XAA and 2282del4 del.

Taking into account the obtained data about the FLG gene polymorphism influence not only on the mophrofunctional characteristics of the skin, but also on the development of atopy in general; the attention was paid to the presence of a concomitant allergic pathology in the examined children (Table 1). The general level of a concomitant allergic pathology reached (45.0 ± 7.0)% in children of the main group and (55.1 ± 6.1)% in children of the control group, p>0.05.

*Table 1*

The spectrum of a concomitant allergic pathology in children with AD considering the FLG gene polymorphism

<table>
<thead>
<tr>
<th>Concomitant pathology</th>
<th>Main group (n = 51)</th>
<th>Control group (n = 60)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>M ± m, %</td>
<td>n</td>
</tr>
<tr>
<td>Food allergy</td>
<td>18</td>
<td>35.3 ± 6.7</td>
<td>27</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>20</td>
<td>38.0 ± 6.8</td>
<td>17</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>3</td>
<td>5.7 ± 3.2</td>
<td>4</td>
</tr>
<tr>
<td>Other allergic diseases</td>
<td>11</td>
<td>20.9 ± 5.7</td>
<td>21</td>
</tr>
</tbody>
</table>
According to the presented results, there was not significant difference between the groups in the spectrum of concomitant pathology, but the children of the main group were diagnosed bronchial asthma more frequently, which can be explained by early onset of AM in patients with FLG polymorphism and the earlier onset of BA, as the next step of the atopic reaction cascade. It should be underlined that the number of children with concomitant allergy, such as food allergy, urticaria and Quincke's edema increased in the control group. Obviously, the patients of this group had more significant IgE – dependent atopic mechanisms, which indicates the active involvement of immunological processes in the implementation of AM.

It was determined, that the average value of the SCORAD index in the main group of patients was (52.8 ± 7.5) scores, and in the control – (33.0 ± 4.0) scores, p<0.05; damaged area – (52.1 ± 13.2)% and (19.5 ± 10.2)%, respectively, p <0.001; the severity of objective signs – (10.2 ± 1.7) scores and (7.2 ± 1.3) scores, respectively, p>0.05; itching intensity – (3.5 ± 0.5) scores and (2.3 ± 1.1) scores, respectively, p>0.05; sleep disorders – (3.2 ± 0.6) scores and (1.8 ± 0.6) scores, respectively, p>0.05. So, when the polymorphism R501X Aa, 2282 del4 is present, SCORAD data are deviated more significantly, and the most sensitive component was the damaged area.

The level of markers of allergic inflammation was not significantly affected by progression of AM, in a total cohort, eosinophilia was documented in (14.6 ± 3.74)%; an increase in serum IgE concentration – in (61.8 ± 5.2)% of children.

When determining the FLG polymorphism effect on the clinical course of AD, the presence of the associative relationship was established with the following indicators: the early onset of the disease – $\chi^2 = 33.2$, mostly severe course – $\chi^2 = 16.2$, severe skin dryness – $\chi^2 = 22.6$, predominant sensitization to fungi – $\chi^2 = 10.6$ and house dust mites – $\chi^2 = 12.2$, violation of the skin microbiome – $\chi^2 = 7.8$.

The implementation of the atopic march significantly affects the quality of life of the patient because of the need to keep numerous restrictions in everyday life and nutrition. Since the maximum effect of treatment is determined by achieving control over chronic allergic diseases, the quality of life index can be used not only to
determine the psychological state of the patient but also to assess the course of allergic diseases and to determine the effectiveness of therapeutic measures.

To assess the impact of atopic diseases on the quality of life of the child with the help of adapted questionnaires, the psychological state of patients was assessed. The quality of life index in the filaggrin-associated atopic march was $(25.6 \pm 1.9)$, and in the comparison group $(20.4 \pm 2.1)$ scores. As the filaggrin-associated atopic march is characterized by the early onset and the more severe course, it is clear that patients experience greater psychoemotional stress.

**Conclusions.** So, the polymorphism of the filaggrin gene can serve as an informative marker for predicting the development of atopic march in children with AD.

Hypersensitivity of the skin under conditions of genetically determined deficiency of FLG derivatives is accompanied by increased dryness of the skin due to transcutaneous loss of moisture in the *stratum corneum*, which affects the barrier functions and leads to excessive penetration of allergens, formation of sensitization and AM with transformation into combined allergic pathology of AD with AR in $(5.7 \pm 3.2)\%$, BA – in $(38.0 \pm 6.8)\%$.

Early manifestation of atopic march under conditions of genetic polymorphism R501XAA and 2282de4AA in children with the filaggrin-associated atopic dermatitis requires restoration of the epidermal skin barrier to prevent transcutaneous penetration of allergens and early sensitization of the body and for prevention of allergic pathology progression.

**Prospects for further research.**

The study of the peculiarities of the course of AD under conditions of the filaggrin protein defect is a promising area of further research, which will determine the risk of AM progression with the development of AR and BA in children and develop differentiated prophylactic and treatment algorithms.

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