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Detection of Allelic Variants and Association of Filaggrin Gene Polymorphism Genotypes in Dermatomycoses Among Children in the Uzbek Population

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Abstract The pathogenesis of fungal skin diseases highlights the importance of the skin's barrier function - the genes encoding epidermal proteins responsible for forming the epidermal barrier. This study aimed to investigate allelic variants and associations of the 2282del4 polymorphism genotypes in the FLG gene among children with fungal skin and scalp diseases in the Uzbek population. Materials and Methods: We examined 16 children aged 1 to 18 with fungal diseases caused by dermatophytes. Clinical, immunological, molecular genetics and statistical analyses were conducted for all children. Results: Molecular genetic analysis of the frequency distribution of the 2282del4 polymorphism genotypes in the FLG gene revealed an increased heterozygous variant of the FLG gene in the primary group of children with fungal diseases in 31.3% (5/16) of cases, whereas it was not detected in the control group ($\chi2$=10.52, P<0.005; OR=29.2; 95% CI 1.49 - 570.6). The mutant homozygous variant of the 2282del4 polymorphism in the FLG gene was not detected in both groups ($\chi2$=10.52, P<0.005; OR=1.8; 95% CI 0.04 - 97.5). Conclusion: The heterozygous genotype variant of the 2282del4 polymorphism in the FLG gene is a significant marker of an increased risk of developing fungal diseases caused by Trichophyton and Microsporum dermatophytes among children in the Uzbek population ($\chi2$=10.52, P<0.005; OR=29.2; 95% CI 1.49 - 570.6). The functionally favorable genotype of the 2282del4 polymorphism in the FLG gene is a significant protective marker against pathology ($\chi2$=10.5, P<0.005; OR=0.03; 95% CI 0.0-0.67).

Keywords Dermatomycoses, Trichophytosis, Microsporia, Filaggrin gene, 2282del4 FLG gene polymorphism, Genetics

1. Introduction

In dermatological practice, fungal skin diseases remain relevant, given the continuous rise in their prevalence, particularly among children. Among the causative agents of fungal skin diseases, fungi from the Trichophyton, Microsporum, and Malassezia genera are most commonly observed [1,5]. The high contagion of mycosis agents, the development of infiltrative-purulent forms, chronic courses, and resistance to antifungal therapy necessitate a more profound exploration of the etiopathogenetic mechanisms of mycotic skin infections.

A critical aspect in the pathogenesis of fungal diseases is the skin's barrier function. Studies have shown that disruptions in the skin's barrier function in various skin diseases, particularly atopic dermatitis, are attributed to mutations in the gene encoding filaggrin (FLG), part of the epidermal differentiation complex [3,4,7,9-12].

Filaggrin is the primary protein responsible for epidermal cell differentiation and the execution of its barrier function. In the stratum corneum, filaggrin molecules, rich in arginine, undergo deimination, converting positively charged arginine residues into neutral citrulline. These molecules disassociate from keratin and degrade into various amino acid components, including pyrrolidone carboxylic acid and urocanic acid [2,3,4].

In cases where filaggrin is reduced, as in atopic dermatitis, or absent, as in ichthyosis, the skin's barrier quality deteriorates due to the stratum corneum's inability to retain moisture [4,7,9].

This has sparked significant interest in investigating the filaggrin gene (FLG) in developing fungal skin infections in children. The search for genetic factors will help uncover the primary mechanisms underlying the pathogenesis of fungal diseases [6].

Objective:

The study aimed to explore allelic variants and associations of the 2282del4 polymorphism genotypes in the FLG gene among children with Trichophytosis in the Uzbek population.

2. Materials and Methods

We examined 16 children aged 1 to 18 years with fungal skin and scalp diseases, with 10 (62.5%) girls and 6 (37.5%)

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boys. Clinical, immunological, molecular-genetic, and statistical studies were conducted for all children. The diagnosis of skin mycosis was made by ICD-10 (International Classification of Diseases, 10th revision) codes, with the primary code being B35.0 [8].

Molecular-genetic biomaterials (DNA) examinations were carried out at the "Geno-technology" LLC. The study involved DNA samples from children with fungal skin diseases, who constituted the primary group, and healthy children without fungal skin diseases (the control group). Genetic research was performed following informed consent from the patients. DNA samples were extracted from peripheral blood lymphocytes using a modified methodology. The concentration and purity of the isolated DNA were assessed by measuring the optical density of DNA-containing solutions at wavelengths of 260 and 280 nm against TE (Tris-EDTA) on a NanoDrop 2000 spectrophotometer (USA).

Genotyping of the FLG gene's 2282del4 polymorphism was performed using a real-time PCR amplifier Rotor-Gene 6000 Model 65H0-100 (Australia) with the test system from "Syntol" Cat. No. - NP_555_100_RG (Russia), following the manufacturer's instructions. Statistical analysis of the results was conducted using the statistical software package "OpenEpi 2009, Version 2.3."

Molecular-genetic data, including an assessment of the deviation of the studied DNA polymorphism distributions from the Hardy-Weinberg equilibrium (HWE), were analyzed using the "GenePop" (Genetics of Population) software.

Allele and genotype frequencies (f) were calculated using the formula:

$$f = n/2N \text{ and } f = n/N,$$

where n is the frequency of the variant (allele or genotype), and N is the sample size.

Allele frequencies were calculated using the formula:

$$P = (2N|+N2)/2N,\ q = (2N3+N2)/2N,$$

where P represents the frequency of allele A, q is the frequency of allele a, and N is the total sample size (N=N|+N2+N3), where N|, N2, N3 are the counts of individuals with genotypes AA, Aa, and aa, respectively.

To calculate the odds ratio (OR) with a 95% confidence interval (CI), $\chi 2$, and p-values, the statistical software package "OpenEpi 2009, Version 2.3" was used.

The relative deviation of observed heterozygosity from expected heterozygosity (D) was calculated using the formula:

$$D = (hobs-hexp)/hexp,$$

where hobs and hexp represent the observed and expected heterozygosity, respectively.

The prognostic efficiency (AUC - area under the curve) of the studied genetic markers was determined using the standard formula:

$$AUC = (Se + Sp)/2,$$

Where Se and Sp are the sensitivity and specificity of the genetic marker, respectively, if the AUC value is <0.5, the marker is a random classifier; AUC=0.5–0.6 indicates a poor classifier; AUC=0.6–0.7 is a moderate classifier; AUC=0.7–0.8 is a good classifier, and AUC>0.8 represents an excellent classifier.

Statistical significance was accepted at $p<0.05$.

3. Results

In terms of clinical forms, among the 16 children, 14 were diagnosed with smooth skin trichophytosis and trichophytosis of the scalp, while 2 had microsporia of the smooth skin. Among the 14 children with trichophytosis, five were diagnosed with the superficial form of smooth skin trichophytosis, and 9 had the infiltrative-purulent form of scalp trichophytosis. All children were included in the combined group for laboratory analysis. The results of the molecular-genetic investigations of the FLG gene's 2282del4 polymorphism are presented in Table 1.

As evident from the table, a comparative analysis of allele and genotype frequency distribution of the 2282del4 FLG gene polymorphism among 60 DNA samples from 30 healthy children revealed the presence of the normal gene allele in 100% of cases (60/60, N=60, n=60), whereas in the primary group of children with trichophytosis, among 114 DNA samples (N=114, n=32), the normal allele was identified in 84.4% (27/32), which was 1.2 times lower compared to the control group (*$\chi 2$=9.91, P<0.002; OR=0.04; 95% CI 0.0-0.77). Meanwhile, the mutant allele of the 2282del4 FLG gene was detected in 15.6% of cases (5/32) in the primary group of children with trichophytosis, whereas it was not observed in the control group (*$\chi 2$=9.92, P<0.002; OR=24.2; 95% CI 1.29 - 453.2).

Table 1. Frequency distribution of allele variants and 2282del4 FLG gene polymorphism in children with smooth skin trichophytosis and the control healthy group

№	Group	Allele frequency				Genotype distribution frequency					
		normal		mutant		normal		heterozygotes		Homozygous mutant	
		n	%	n	%	n	%	n	%	n	%
1.	Main group n=16 (32)	27	84,4	5	15,6	11	68,7	5	31,3*		
2	Control group n=30 (60)	60	100			30	100				

N - the number of patients examined; *n - the number of chromosomes examined;
* - a confidence indicator about the control group (P<0.05)

Table 2. Differences in the Frequency of Allele and Genotype Occurrence of the 2282del4 FLG Gene Polymorphism in the Primary and Control Groups

Alleles and Genotypes	Number of Investigated Alleles and Genotypes		Statistical Differences
	Study Group	Control Group	
Allele (Normal)	27	60	χ^2=9.92, P<0.002;OR=24,2;95%CI 1.29 - 453.2
Allele (Mutant)	5	0	
Normal Genotype	11	30	χ^2=10.5, P<0.005; OR=0,03;95%CI 0.0- 0.67
Heterozygote	5	0	χ^2=10, 52, P<0.005;OR=29,2;95%CI 1.49 – 570,6
Mutant Genotype	0	0	χ^2=10.52, P<0.005;OR=1,8;95%CI 0.04 – 97,5

The data obtained in our study indicate a heightened association between the mutant allele of the 2282del4 FLG gene polymorphism and atopic dermatitis, the risk of which increases by 24.2 times when possessing this genetic determinant (OR=24.2).

The analysis of the distribution of genotypic variants of the 2282del4 FLG gene polymorphism revealed a predominance of the homozygous genotype for the "wild-type" allele in the healthy children of the control group - 100% (30/30). In the primary group of children with trichophytosis, this genotype accounted for 68.7% (11/16) of cases, which was 1.5 times lower compared to the control group (χ2=10.5, P<0.005; OR=0.03; 95% CI 0.0-0.67). The heterozygous variant of the 2282del4 FLG gene polymorphism was found in the primary group of children with trichophytosis in 31.3% (5/16) of cases, while it was not detected in the control group (χ2=10.52, P<0.005; OR=29.2; 95% CI 1.49 – 570.6). The mutant homozygous variant of the 2282del4 FLG gene polymorphism was not found in either group (χ2=10.52, P<0.005; OR=1.8; 95% CI 0.04 – 97.5) (Table 1).

Despite the small sample sizes of the studied groups, the obtained results hold significant value in terms of disease risk prediction. An essential aspect of studying polymorphic genes potentially associated with the development and pathogenesis of diseases is the analysis of expected and observed genotype frequencies of the studied polymorphisms and the congruence of frequency distribution with the Hardy-Weinberg equilibrium (HWE).

Table 3. Expected and Observed Frequency Distribution of Genotypes by HWE for the 2282del4 FLG Gene Polymorphism in the Study Group of Children with Trichophytosis

Genotypes	genotype frequency		χ^2	P
	Observable	expected		
Norm	68,7	71,2	0,013	0,78
Hetero	31,3	26,4	0,146	
Mutant	0	2,44	0,391	
Total	100,00	100,00	0,549	

Analysis of the genotype frequency distribution parameters according to the Hardy-Weinberg equilibrium (HWE) for the 2282del4 FLG gene polymorphism in the study group of children with tinea capitis revealed an increase in the expected frequencies of favorable genotypes by 1.04 times - 71.2% compared to the observed frequencies of 68.7%. In contrast, heterozygous variants decreased by 1.2 times, accounting for 26.4% compared to the observed 31.3%. Notably, there was an increase in the expected frequencies of often unfavorable homozygous genotypes, which accounted for 2.4% of cases.

The distribution of genotype frequencies of this polymorphism also showed significant differences between the study group and the comparison group in the overall sample ($p < 0.05$). Associations were identified with "functionally unfavorable" heterozygous genotypes ($\chi2 = 8.738$, $p < 0.01$; OR = 15.09; 95% CI 0.86 - 265.5) and mutant homozygous genotypes ($\chi2 = 10.52$, $p < 0.005$; OR = 1.8; 95% CI 0.04 - 97.5) in the development of dermatomycoses caused by dermatophytes.

Thus, the heterozygous genotype of the 2282del4 FLG gene polymorphism represents a significant marker of increased risk for the development of dermatomycoses caused by dermatophytes, particularly those of the Trichophyton and Microsporum genera, in Uzbek children ($p < 0.005$). The functionally favorable genotype of the 2282del4 FLG gene serves as a reliable protective marker against the development of pathology ($\chi2 = 10.5$, $p < 0.005$; OR = 0.03; 95% CI 0.0-0.67).

The obtained data are essential for predicting the risk of fungal diseases among the Uzbek child population. Genotyping can be used for identifying children in families with a history of such diseases who are predisposed to developing fungal infections, allowing for targeted primary prevention. The research is ongoing.

4. Conclusions

1. Molecular-genetic analysis of the frequency distribution of 2282del4 FLG gene alleles in children with fungal diseases revealed the presence of the mutant allele variant in 15.6% of cases (5/32) ($\chi2$=9.92, p<0.002; OR=24.2; 95% CI 1.29 - 453.2).
2. Heterozygous variants of the 2282del4 FLG gene polymorphism were detected in 31.3% of cases (5/16) in the study group of children with trichophytosis, whereas none were found in the control group ($\chi2$=10.52, p<0.005; OR=29.2; 95% CI 1.49 – 570.6). Mutant homozygous variants of the 2282del4 FLG gene polymorphism were not identified in either group ($\chi2$=10.52, p<0.005; OR=1.8; 95% CI 0.04 – 97.5).
3. Heterozygous genotype variants of the 2282del4 FLG

gene polymorphism are a significant marker of increased risk for the development of fungal diseases caused by dermatophytes of the Trichophyton and Microsporum genera in Uzbek children ($\chi 2=10.52$, $p<0.005$; OR=29.2; 95% CI 1.49 – 570.6). Functionally favorable genotype 2282del4 FLG gene variants serve as reliable protective markers against the development of pathology ($\chi 2=10.5$, $p<0.005$; OR=0.03; 95% CI 0.0-0.67).

5. Study Limitations

Within the scope of this study, no significant limitations that could impact the accuracy and generalizability of the obtained results have been identified.

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Conflict of Interest

The authors of this article confirm the absence of any conflicts of interest that could influence the research findings.

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