

# The prevalence of mutations in the gene encoding filaggrin in the population of Polish patients with atopic dermatitis

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## Abstract

**Introduction:** The genetic background of atopic dermatitis (AD) is complex, involves many genes and their participation varies in varied populations, and depends on the intensity and course of a disease. Changes in the nucleotide sequence of the *FLG* gene and a reduced number or a deficit of the functional product of processed profilaggrin can be one of risk factors for atopic dermatitis.

**Aim:** To determine the prevalence of R501X and 2282del4 mutations of the *FLG* gene in patients with AD.

**Material and methods:** The studied group included 60 patients with clinically diagnosed AD, and the control group included 61 healthy volunteers. The study protocol included collection of biological material for tests, DNA isolation and evaluation of its quality and quantity, and PCR amplification of the isolated genetic material.

**Results:** In the studied group, both changes in the nucleotide sequence of the *FLG* gene were detected and in the control group no tested mutations were detected. In 18 (30%) patients with AD, 22 mutations (4 heterozygous and 1 homozygous ones of R501X and 10 heterozygous and 7 homozygous ones of 2282del4) were detected.

**Conclusions:** A high rate of mutations of the *FLG* gene in patients with clinically diagnosed AD and pathologically dry skin was observed in the studied population. The 2282del4 mutation occurred more often than R501X.

**Key words:** dry skin, atopic dermatitis, mutations, filaggrin.

## Introduction

Epidermal proteins, in particular filaggrin (*filament aggregation protein*), one of markers of terminally differentiating keratinocytes, play a significant role in the process of keratinization, formation and normal functioning of the epidermal protective layer. Abnormalities at different stages of its production and further decomposition are a cause of disturbances in the process of terminal cell differentiation, increased permeability of the epidermal barrier, resulting in dry skin that is a key diagnostic criterion of atopic dermatitis (AD). Apart from complex interactions between exogenous and immune factors, genetic conditions also play a role in the development

of AD, and inheritance depends on many genes. However, genetic background of this dermatosis is complex, includes many genes and their participation in different populations varies, and also depends on the intensity and the course of this disease [1–5]. Among others, polymorphisms/mutations in a gene group forming the epidermal differentiation complex (EDC) are responsible for genetically conditioned damage to the dermal barrier in the course of AD. Changes in a nucleotide sequence of the *FLG* gene located on chromosome 1q21, namely a reduced number or a deficit of the functional product of processed profilaggrin can be one of risk factors for ichthyosis and simultaneously constitute one of risk factors for AD and moreover, they might also correlate with

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a phenotype and a clinical course of the disease [1, 2, 6–8]. Reduced expression of filaggrin due to structural changes in the *FLG* gene has been confirmed in pathologically changed and intact skin of patients with AD [9]. Two most frequent *FLG* mutations in European population, R501X and 2282del4, first described by Palmer in 2006, were studied, because both of them are highly predisposing to development of ichthyosis vulgaris as well as to AD.

### Aim

The objective of the work was to determine the prevalence of R501X and 2282del4 mutations of the *FLG* gene in patients with AD.

### Material and methods

The analysis of prevalence of R501X and 2282del4 mutations in the gene encoding filaggrin was performed in 121 adults.

The study protocol was approved Ethical Committee of Collegium Medicum in Bydgoszcz (KB 536/2010 dated 3 December 2010).

The studied group included 60 (49.59%) patients with AD and pathologically dry skin aged between 18 and 61 years (mean age: 29.92 years).

The control group included 61 (50.41%) healthy volunteers without symptoms of dry skin in whom dermatologic and allergic conditions were excluded, aged between 18 and 76 years (mean age: 42.41 years).

The material for analysis included whole peripheral blood collected from study participants by qualified medical personnel into 2-ml tubes with EDTA (1 mg/ml). Identical composition of the reacting mixture and individually chosen primers were used for each reaction and for both mutations.

1. *FLG* R501X 5' – ACA GCC TGA CTC TGC CCA TG – 3' (forward)
2. *FLG* R501X 5' – GCA CTT CTG GAT CCT GAC TG – 3' (reverse)
3. *FLG* 2282del4 5' – TCC CGC CAC CAG CTC C – 3' (forward)
4. *FLG* 2282del4 5' – TG GCT CTG CTG ATG GTG A – 3' (reverse).

The study protocol included collection of biological material for tests, DNA isolation and evaluation of its quality and quantity, and then PCR amplification of the isolated genetic material was performed. After multiplication, the PCR products were digested with a restrictive enzyme and results were visualized in the PAA gel.

### Statistical analysis

The statistical analysis was performed with the statistical software Statistica 10.0 by StatSoft®. Quantitative variables (subjects' age) were described with the follow-

ing statistics: number (*N*), arithmetic mean (*M*), standard deviation (*SD*), median (*Me*), minimum (*Min.*) and maximum (*Max.*). The analysis of a qualitative variable correlation was performed with the  $\chi^2$  test. For 2 x 2 tables and theoretical numbers below 5, the Yates' correction was used. The test likelihood at  $p < 0.05$  was considered to be statistically significant and marked with “\*\*”. The analysis was also supplemented with calculated odd ratios (OR).

### Results

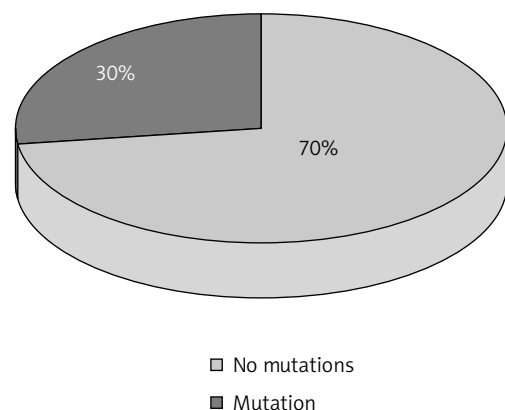
In the studied group both changes in a nucleotide sequence of the *FLG* gene (Figure 1) were detected and in the control group, no tested mutations were detected.

In 18 (30%) patients with AD, 22 mutations (4 heterozygous and 1 homozygous ones of R501X and 10 heterozygous and 7 homozygous ones of 2282del4) were detected (Figure 1).

In 4 patients at the same time both genetic variants were present (4 heterozygous ones of R501X and 2 heterozygous and 2 homozygous ones of 2282del4) (Table 1, Figure 2).

In the case of the R501X mutation, 4 (6.67%) heterozygotes and 1 (1.67%) homozygote were observed, and differences with regard to the total prevalence of heterozygotes and homozygotes for nonsense mutation between the AD group and the control group were close to the statistical significance ( $p = 0.0706$ ). In 61 subjects from the control group (100.00%) and 55 from the studied group (91.66%), a normal genotype with regard to this mutation was demonstrated (Table 2, Figures 3, 4).

When analyzing the prevalence of the 2282del4 mutation, the presence of 10 (16.67%) heterozygotes and 7 (11.67%) homozygotes was observed. A performed statistical analysis revealed significant differences between the total prevalence of heterozygous and homozygous genotypes as compared to the control group ( $p < 0.0001$ ). A normal genotype with regard to this mutation was



**Figure 1.** Prevalence of the *FLG* gene mutations in patients with AD

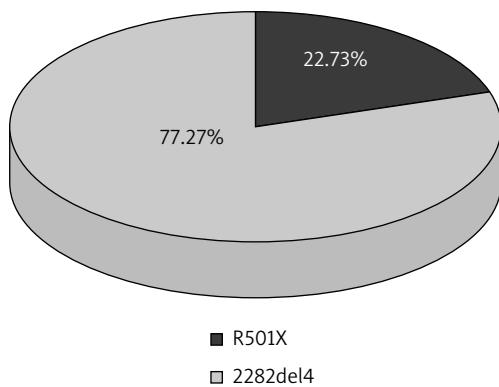
**Table 1.** Patients with AD and pathologically dry skin in whom R501X and/or 2282del4 mutations were observed

No.	Phenotype	Age	Gender	R501X		2282del4	
				Heterozygotes	Homozygotes	Heterozygotes	Homozygotes
1	m	28	F			+	
2	mr	24	F	+		+	
3	s	21	F	+			+
4	s	35	F				+
5	m	22	F			+	
6	m	28	M			+	
7	m	40	F			+	
8	m	26	F			+	
9	m	27	F			+	
10	m	24	M			+	
11	mr	18	F	+		+	
12	s	56	M		+		
13	s	56	M				+
14	s	37	F				+
15	s	51	F				+
16	s	56	F	+			+
17	s	23	F				+
18	m	29	F			+	

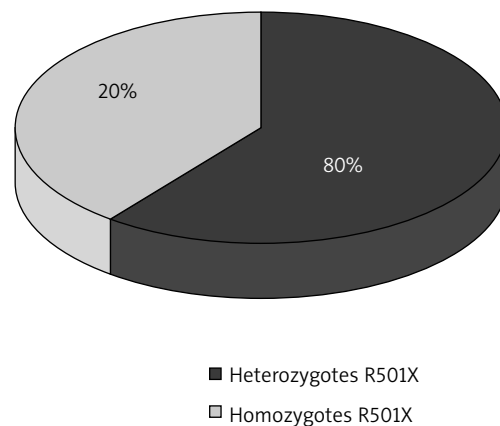
Phenotype: m – mild, mr – moderate, s – severe, gender: F – female, M – male.

**Table 2.** Correlation between the prevalence of the R501X mutation and AD development

R501X	Control group		Studied group		OR*	P-value
	1		2			
	N	%	N	%		
Total	61	100.0	60	100.0	5.56	0.0706
Normal genotype	61	100.0	55	91.6		
Heterozygotes	0	0.0	4	6.6		
Homozygotes	0	0.0	1	1.6		



**Figure 2.** Prevalence of R501X and 2282del4 mutations in patients with AD



**Figure 3.** Prevalence of R501X heterozygotes and homozygotes in patients with AD

**Table 3.** Correlation between the prevalence of the 2282del4 mutation and AD development

2282del4	Control group		Studied group		OR*	P-value	
	1		2				1-2
	N	%	N	%			
Total	61	100.0	60	100.0	24.15	< 0.0001*	
Normal genotype	61	100.0	43	71.6			
Heterozygotes	0	0.0	10	16.6			
Homozygotes	0	0.0	7	11.6			

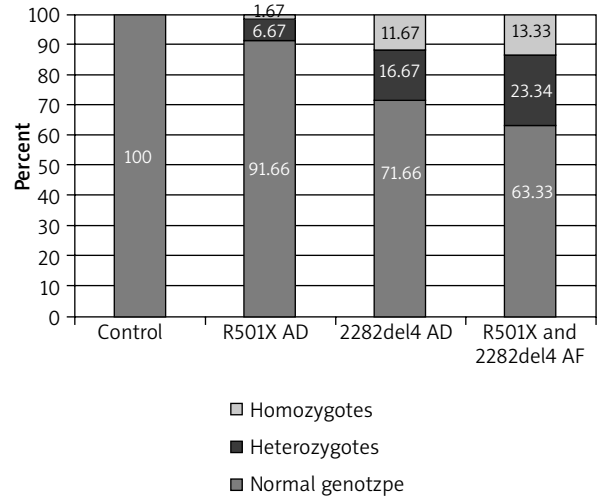
present in 43 subjects in the studied group (71.66%) and 61 subjects in the control group (100.00%) (Table 3, Figures 4, 5).

When considering the total prevalence of the R501X substitution and the 2282del4 deletion in the studied group, 14 (23.34%) heterozygotes and 8 (13.33%) homozygotes were observed, and a statistical analysis performed also indicated significant differences between the total prevalence of heterozygous and homozygous genotypes as compared to the control group ( $p < 0.0001$ ) (Table 4, Figures 4, 6).

Based on the results obtained, it was concluded that the presence of the deletion increases the likelihood of AD 24 times (OR = 24.15), and its combination with the substitution increases this risk more than 35 times (OR = 35.32).

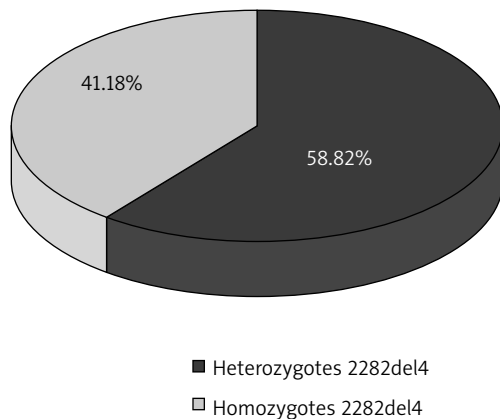
**Discussion**

According to the authors, *FLG* gene mutations predispose to ichthyosis and AD that often coexists with ichthyosis. Our own observations confirm a semidominant inheritance of AD determined by Palmer (high penetration in *FLG*-null homozygotes/complex heterozygotes, low in heterozygotes) [1, 2, 7, 9]. A molecular analysis of the genetic material collected from patients confirmed

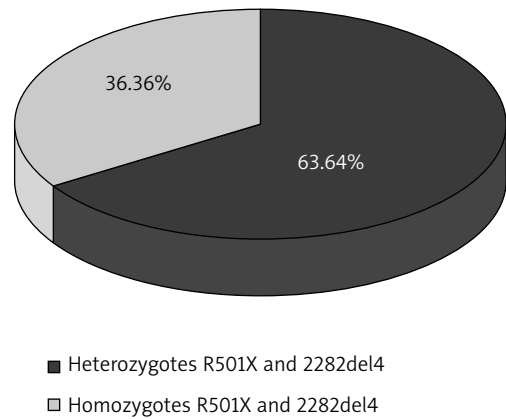


**Figure 4.** Prevalence of R501X and 2282del4 mutations in the control group and in patients with AD

their participation in epidermal barrier dysfunctions. Probably the first dermal lesions are located in the areas where the stratum corneum, namely the protective barrier is the thinnest, what additionally makes it easier for irritants and allergens to pass [10]. Kezic *et al.* conclude that abnormalities in a process of filaggrin formation and



**Figure 5.** Prevalence of 2282del4 heterozygotes and homozygotes in patients with AD



**Figure 6.** Total prevalence of heterozygotes and homozygotes in patients with AD

**Table 4.** Correlation between the prevalence of the *FLG* mutation and AD development

R501X and 2282del4	Control group		Studied group		OR*	P-value	
	1		2				1-2
	N	%	N	%			
Total	61	100.0	60	100.0	35.32	< 0.0001*	
Normal genotype	61	100.0	38	63.3			
Heterozygotes	0	0.0	14	23.3			
Homozygotes	0	0.0	8	13.3			

its further decomposition are also a cause of dry skin [11]. On the other hand, Weidinger *et al.* demonstrated a correlation between *FLG* gene mutations and exogenous AD accompanied by increased IgE levels and positive spot tests [12]. However, the authors did not observe any significant correlations between these changes and endogenous AD, whereas Marenholtz *et al.* indicated a growing predisposition for EADS and IADS as well [13, 14]. According to Stemmler *et al.*, R501X and 2282del4 mutations are present in 10.3% of patients in whom AD developed at the age above 2 years, and in 12.1% of patients in whom this condition developed earlier. The presence of at least one mutated allele was confirmed in 21.3% of patients and as a result it was possible to draw a hypothesis assuming a correlation between *FLG* gene mutations and an early start of AD [15]. However, neither Weidinger *et al.* nor Lesiak *et al.* demonstrated such a correlation [2, 14]. In our work we did not perform a detailed assessment of the correlation between a genetic defect in the *FLG* gene and an early start of AD because information about when the disease had started was only and exclusively taken from a medical history collected earlier, as subjects below 18 years old were excluded. Both mutations predispose to the development of atopic dermatitis with a moderate or severe, long-term and recurrent course [16, 17]. When verifying a correlation between these mutations and disease severity Lesiak *et al.* indicated that the 2282del4 mutation increases the risk of a moderate and severe form of AD more than two-fold what complies with the Morar's reports [2, 18]. Additionally, Ekelund *et al.* noticed that *FLG-null* variants (homozygous mutations) are more prevalent in the case of a severe AD phenotype [19]. Results of studies by Marenholtz *et al.* and Weidinger *et al.* indicate a significant correlation between nonsense mutations in the filaggrin gene and AD, and this relationship regards approximately 30% of patients with this disease [13, 14]. An identical result, approximately 30%, was also obtained in our own study; however, for both mutations together. Thanks to the analysis of correlations between said mutations and a phenotype of atopic dermatitis, the Sandilands' team was able to present a thesis that R501X as well as 2282del4 are present first of all in patients in whom dominant features of the clinical manifestation include dry skin, keratosis pilaris and increased crease

formation on the palmar surfaces of the hands [20]. In our own study, a correlation with dry skin was first of all demonstrated, what probably is a result of the fact that symptoms of dry skin were present in all patients with AD. Lack of a correlation between studied mutations and ichthyosis is a result of patients' selection as the study did not include patients with this genodermatosis.

## Conclusions

A high rate of mutations of the *FLG* gene in patients with clinically diagnosed AD and pathologically dry skin was observed in the studied population. The 2282del4 mutation was present more often than R501X in the studied population.

## Conflict of interest

The authors declare no conflict of interest.

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