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The Role of the *Ctla4* Gene in Psoriasis of Different Severity

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ABSTRACT

Objectives. To determine the role of the genetic genotypes of the *CTLA4* gene (49A/G) in psoriasis of different severity.

Material and methods.60 patients with psoriasis of different severity were examined. Two groups were identified: the first (n=30) – moderately severe psoriasis, the second (n=30) – severe psoriasis. Comprehensive examination included the determination of the level of nitric oxide (NO), lipid peroxidation products (diene conjugates, malondialdehydes, and antioxidant activity (superoxide dismutase, SOD), cytokines (interleukin 1β (IL-1β), tumor necrosis factor alpha (TNF-α) , IL-10) in blood plasma.molecular genetic study of polymorphism (*49A/G*) of the *CTLA4* gene by real-time PCR (CFX96 Touch System, USA).

Results. During the study it was established that the psoriasis of varying severity is characterized by a significant disturbance in the homeostasis system in the form of a significant activation of lipid peroxidation (LPO), increased secretion of nitric oxide and various types of cytokines. The presence of conditionally "pathogenic" genotypes *G/G* and *A/G* of the *CTLA4* gene,the course of psoriasis is more severe: pronounced oxidative stress, significant inhibition of antioxidant activity, rapid development of nitrogenous intoxication, excessive pro-inflammatory response and inhibition of the anti-inflammatory component of the immune system.

Conclusions. Our data allow us to make another conclusion that in order to select the optimal diagnostic and therapeutic algorithm for psoriasis of varying severity, it is necessary to be guided by the information on genetic analyzes of the *CTLA4* gene polymorphism (*49A/G*). This will make it possible to personalize the approach to the treatment of patients with psoriasis of varying severity, which has recently been given the main role by modern dermatovenerology.

Keywords:ген, ctla4, *49A/G*,psoriasis, LPO, SOD, NO

INTRODUCTION

In the last decade, psoriasis – is a chronic multifactorial systemic inflammatory pathology, accompanied by damage to the skin and a number of other organs, is an urgent issue in dermatological practice. This is due to the annual increase in the incidence (1–8% of the world occurs), high prevalence (up to 40% among dermatological diseases), affects people of working age, the fascination with cases of disability and long-term disability, large financial costs [1].

Psoriasis is usually not accompanied by death of patients, but causes a significant deterioration in self-esteem, quality of life of patients, their social adaptation [2].

The pathogenesis of psoriasis is based on the deviation of the immune cytokine reaction, especially Th1, Th17-type, which, with the dominance of cytokines: interleukins 1, 6, 17, 22, tumor necrosis factor α, etc., synthesized by CD4 + -activated lymphocytes, leads to the proliferation of keratinocytes, epidemiological proliferation, neovascularization, development of psoriatic plaque [3].

Cytotoxic T-lymphocyte glycoprotein 4 (CTLA4, CD152) is a membrane cell receptor and from the immunoglobulin superfamily, which is located on the surface of T-helpers and transmits an inhibitory signal to T-lymphocytes, leading to inhibition of the immune response [4]. The transformation (*49 A/G*), characterized by the nitrogenous base of adenine (*A*) to guanine (*G*) in the firstexon of the *CTLA4* gene, leads to a change in the organic compound tryptophan (*Thr*) to alanine (*Ala*) in the 17th codon. This mutation results in decreased regulation of T-cell activation and the development of autoimmune reactions [5, 6].

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RESEARCH OBJECTIVES

To determine the role of the genetic genotypes of the CTLA4 gene (49A/G) in psoriasis of different severity.

RESEARCH MATERIALS AND METHODS

A prospective study was carried out in 60 patients with psoriasis of varying severity who were hospitalized at the Dermatovenerologic Dispensary № 3, Moscow, according to the recommendations of the World Medical Association [7].

Molecular genetic analyzes of the examined patients were carried out on the basis of the Department of Genetics of the Moscow State University.

Inclusion criteria: written consent to participate in the work, patients of both sexes, age from 22 to 45 years; individual written informed consent to participate; clinical, laboratory and instrumental verification of the diagnosis of psoriasis. Exclusion criteria: the patient's own refusal to participate; age over 45 and under 22; the presence of severe concomitant somatic, infectious, mental illnesses.

The patients under study were randomized into 2 groups depending on their severity of the disease, established during hospitalization. The first group (comparison, n=30) consisted of patients with moderate psoriasis (age – 30.5 ± 7.89, men were 17 (56.7 %), women – 13 (43.3 %)). The second group (main, n = 35) included patients with severe psoriasis (age – 32.7 ± 8.16, men were 18 (60.0 %), women – 12 (40.0 %)).

To compare the indicators of the homeostasis system of the studied patients with the norm, healthy individuals (n=35) of both sexes at the age from 23 to 52 years were examined.

To identify the severity of psoriasis, the PASI (Psoriasis Area and Severity Index) score was used, which assesses the area and prevalence of skin lesions and the severity of the main psoriatic symptoms when using the “palm” rule. The average PASI score in the patients of the comparison group was 9.15 ± 0.55, and the main one – 28.43 ± 4.71, which is defined as moderate and severe severity [8].

In patients of the 1st group, skin lesions in the form of papular and small plaque lesions of limited areas of the skin with mild hyperemia, slight infiltration, desquamation and mild itching were noted. In patients of the main group, more common skin lesions (papules and large plaques) were recorded against the background of severe hyperemia, significant infiltration and desquamation, and intense itching.

During observation, the study patients were prescribed standard therapy according to the Clinical Recommendations of the Russian Society of Dermatovenereologists (2010), which included desensitizing, hepatoprotective, antihistamine components, vitamins and external agents (anti-inflammatory, keratolytic, glucocorticoid).

Biochemical tests were applied. Determination of the level of nitric oxide (NO), products of lipid peroxidation (diene (DC) conjugates, malondialdehydes (MD), and antioxidant activity (superoxide dismutase, SOD), cytokines (interleukin 1β (IL-1β), tumor necrosis factor alpha (TNF-α), IL- 10) in blood plasma.

Analysis of polymorphic genotypes of the *CTLA4* gene (*49A/G*) was performed using the real-time polymerase chain reaction (CFX96 Touch ™ Real-Time PCR DetectionSystem (USA)).

Statistical processing of the obtained digital results was carried out using the Statistica 13.3 Trial program. Used: Fisher's and Pearson's χ^2 tests, confidence interval (95%, DI), and odds ratio calculation (OR, 95%).

RESEARCH RESULTS

The main pathogenetic component of the development of psoriasis is an excessive intensification of the oxidative phenomenon, which is accompanied by pronounced production of both nitric oxide and endogenous biologically active substances (cytokines) [9, 10].

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In the course of the study, it was found that psoriasis of different severity is characterized by a significant disturbance in the homeostasis system in the form of a significant activation of lipid peroxidation (LPO) processes, increased secretion of NO and various types of cytokines (Table 1).

Table 1 – indicators of the homeostasis system in psoriasis

Indicator	Norm (n=35)	Study groups	Value
DC, conv. un. / mg lipids	211,3±10,7	I (n=30)	**312,5±12,6**
		II(n=30)	**375,2±14,2**[1]
MD, nMol / g protein	2,22±0,13	I (n=30)	**2,98±0,23**
		II(n=30)	**3,78±0,36**[1]
SOD, conv. un.	8,75±0,41	I (n=30)	**6,45±0,35**
		II(n=30)	**5,12±0,45**[1]
NO, μmol/l	26,1±3,45	I (n=30)	**33,6±4,11**
		II(n=30)	**39,9±5,32**[1]
IL-1β, pg / ml	0,95±0,08	I (n=30)	**1,45±0,13**
		II(n=30)	**1,88±0,22**[1]
TNF-α, pg / ml	3,86±0,42	I (n=30)	**5,32±0,62**
		II(n=30)	**6,57±0,78**[1]
IL- 10, pg / ml	13,99±1,25	I (n=30)	**11,3±0,85**
		II(n=30)	**9,67±0,74**[1]

Note. Bold text – statistically significant in relation to the norm at $p<0.05$.[1] – statistically significant in relation to the data of the first group at $p<0.05$.

When studying the processes of lipid peroxidation in patients with psoriasis, their pronounced activation was noted, which indicates their main role in the pathogenesis of pathology [11].

When assessing the intensity of activation of lipid peroxidation processes in psoriasis, it was found that in patients of both the first group and the second, the content of both primary (DC) and secondary (MD) LPO products exceeded the norm by 47.8 and 78.1 and 34.2, and 70.2% ($p <0.05$), respectively. At the same time, a comparative analysis showed that the values of diene conjugates and malondialdehydes in patients of the main group exaggerated the comparison group by 20.4 and 26.8% ($p <0.05$).

It is known that oxidative stress is accompanied by structural and functional disorders of the cell membrane of the main organs and systems, on the one hand, and dysregulation of the immune and systemic response, on the other [12].

The intensification of the general oxidative capacity of blood in patients with psoriasis, as the table shows, leads to inhibition of the antioxidant system [13,14].

SOD activity in moderate and severe psoriasis was decreased relative to the normal group by 26.2 and 41.4 % ($p<0.05$), respectively. At the same time, the results of the comparative test of the first and second groups noted that the concentration of superoxide dismutase in severe disease was 20.6 % higher than the moderate one ($p<0.05$).

The development of oxidative endotoxicosis is accompanied by a large release of nitric oxide, which causes cytotoxic effects and cellular changes [15, 16].

In patients of the first group, the concentration of NO was exceeded by 28.7 % ($p<0.05$). In severe psoriasis, the severity of nitrogenous intoxication was 18.7 % higher than the comparison group ($p<0.05$).

Along with the formation of a pronounced oxidative phenomenon, weakening of the antioxidant system, changes in the pro-inflammatory and anti-inflammatory components of the immune system are recorded (table 1).

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Thus, in the patients of the comparison group, the following disorders were found in comparison with the reference indicators: an increase in the content of interleukin 1β and tumor necrosis factor alpha by 52.6 and 37.8 % ($p<0.05$) and a decrease in interleukin 10 by 18.7 % ($p<0.05$).

In patients of the main group, more pronounced immune disorders were recorded. It was found that the level of IL-1β and TNF-a exaggerated the first group (with moderate form of psoriasis) by 29.6 and 23.4% ($p<0.05$), and the concentration of IL-10 was less by 14.4 % ($p<0.05$).

We studied polymorphic (*49A/A*, *49A/G*, *49G/G*) of the *CTLA4* gene in patients with psoriasis (table 2).

Using real-time polymerase chain reaction analysis, it was recorded that the frequency of genotypes (*A/A*, *A/G*, *G/G*) in the examined patients were as follows: in the comparison group – 23.3, 50.0 and 26.7 %, and in the main one – 10.0, 43.3 and 46.7 %, respectively (table 2).

Table 2 - Frequency of occurrence of *CTLA4* gene polymorphism (*49A/G*) in psoriasis

Groups	Genotype and its frequency, (n,%)			Allele and its frequency, (n,%)	
	A/A	*A/G*	*G/G*	*A*	*G*
Norm (n=35)	20 (57,1)	14 (40,0)	1 (2,9)	27 (77,1)	8 (22,9)
I (n=30)	7 (23,3)	15 (50,0)	8 (26,7)	14,5 (48,3)	15,5 (51,7)
II (n=30)	3 (10,0)	13 (43,3)	14 (46,7)	9,5 (31,7)	20,5 (68,3)

When studying the assessment of the significance of differences in outcomes depending on the impact of a risk factor, it was found that the *A/G* genotype has an average relationship with moderate severity (χ^2=3.9, p=0.04 and OR=3.06 (0.9-9,4)) and a strong connection in severe degree (χ^2=7.09, p=0.008 and OR=6.19 (1.4–25.8)). The genetic variant *G/G* had a strong relationship both in the first group (χ^2=11.0, p=0.001 and OR=27.8 (2.3–216.8)) and in the second – (χ^2=23.6 , p=0.001 and OR=93.3 (8.7-992.4)).

When carrying out molecular genetic studies in psoriasis, a reliable association of disorders of the homeostatic system with the studied genotypes of the *CTLA4* gene was established. This was the basis for dividing patients into subgroups. The first group is divided into subgroups 1 (n=14) and 2 (n=16), the second – into subgroups 3 (n=9) and 4 (n=21).

Table 3 - Correlation of indicators of the hemostasis system with genotypes of the *CTLA4* gene (*49A/G*)

Indicator	Norm(n=35)	Подгруппы	Значение
DC, conv. un. / mg lipids	211,3±10,7	1 (n=14)	**285,0±10,5**
		2 (n=16)	**334,1±9,74**[1]
		3 (n=9)	**293,0±11,4**
		4 (n=21)	**398,2±10,8**[2]
MD, nMol / g protein	2,22±0,13	1 (n=14)	**2,78±0,23**
		2 (n=16)	**3,41±0,19**[1]
		3 (n=9)	**3,05±0,22**
		4 (n=21)	**3,99±0,25**[2]
NO, μmol/l	26,1±3,45	1 (n=14)	**31,2±3,71**
		2 (n=16)	**35,1±4,23**[1]
		3 (n=9)	**36,4±3,84**
		4 (n=21)	**42,3±4,25**[2]
IL-1β, pg / ml	0,95±0,08	1 (n=14)	**1,30±0,11**

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		2 (n=16)	**1,48±0,21**[1]
		3 (n=9)	**1,57±0,16**
		4 (n=21)	**1,95±0,28**[2]
TNF-α, pg / ml	3,86±0,42	1 (n=14)	**4,94±0,45**
		2 (n=16)	**5,55±0,56**[1]
		3 (n=9)	**5,13±0,47**
		4 (n=21)	**6,33±0,85**[2]

Note. Bold text – statistically significant in relation to the norm at $p<0.05$.[1] - statistically significant in relation to the data of the first subgroup at $p<0.05$. [2] - statistically significant in relation to the data of the third group at $p<0.05$

When studying the conjugation of the studied genotypes of the *CTLA4* gene and homeostasis indicators under psoriasis conditions against the background of standard therapy, it was recorded that in the 1st subgroup (n=14), where the frequency of the homozygous conditionally normal genotype (*AA*) was 23,3 %, heterozygous (*AG*) – 30,0 % shows the smallest deviations in the homeostatic. At the same time, they were observed: an increase in the content of LPO products – DC and MDA by 35.0 and 25.2 % ($p<0.05$), a decrease in antioxidant activity by 21.0 % ($p<0.05$), an increase in the concentration of oxide nitrogen by 19.5 % ($p<0.05$), an increase in the secretion of IL-1β and TNF-α by 36.8 and 27.9 % ($p<0.05$) and a decrease in the production of IL-10 by 14.3 % ($p<0.05$).

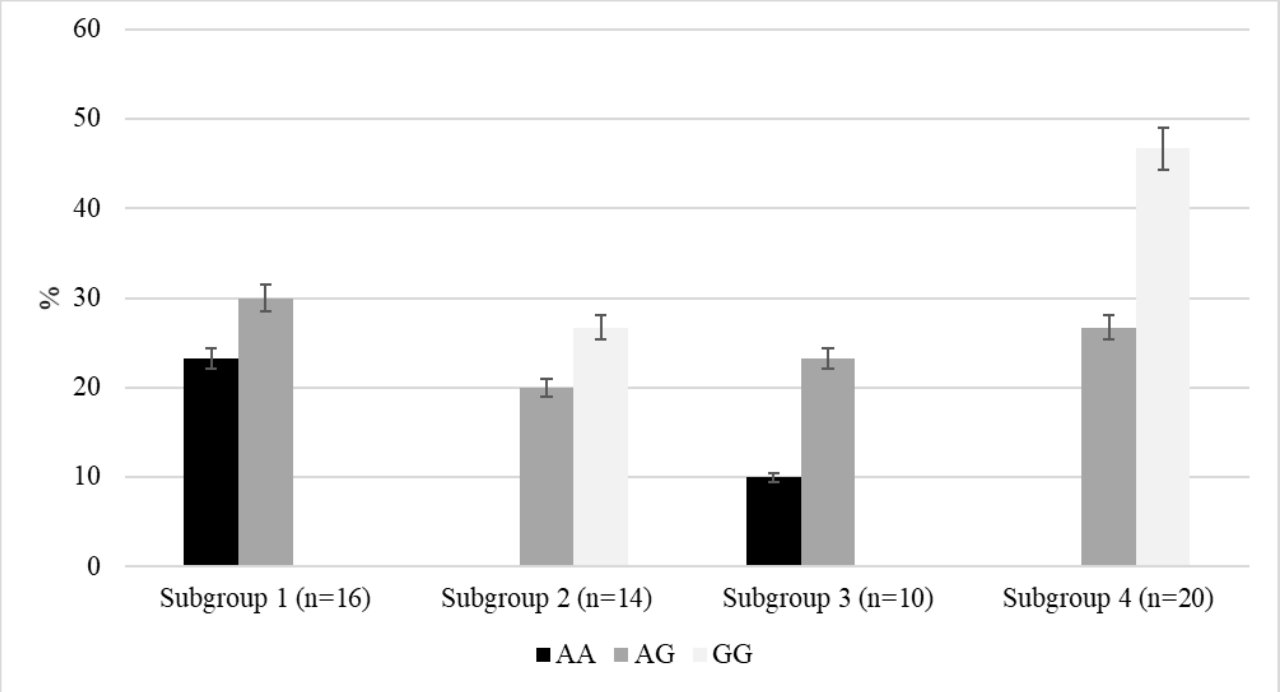

Figure 1. Genetic distribution of the studied patients into subgroups in the association of homeostatic system disorders

In patients of the second subgroup, where opportunistic genotypes were encountered (homozygous (*G/G*) –26,7 %, and heterozygous (*A/G*) –26,7 %) of the *CTLA4* gene, deviations in the hemostasis system were significantly higher than in the first subgroups: DC and MDA - higher by 17.1 and 22.6 % ($p<0.05$), SOD - less by 12.4 % ($p<0.05$), NO - more by 12.6 % ($p<0.05$), IL-1β and TNF-α - higher by 13.8 and 12.4 % ($p<0.05$), and IL-10 – less by 14.4 % ($p<0.05$).

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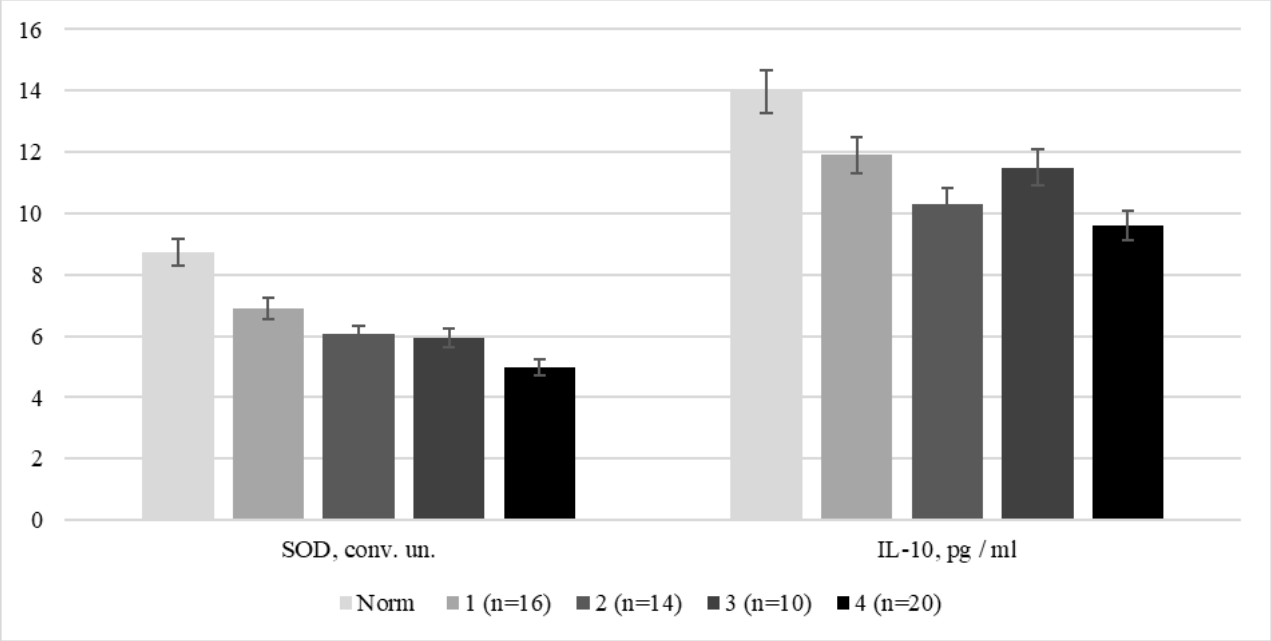

Figure 2. Correlation of homeostasis system parameters (MDA and IL-10) with *CTLA4* (*49A/G*) genotypes

When studying homeostatic parameters in patients with severe psoriasis, it was found that in patients of the 4th subgroup (with polymorphic variants – *G/G* (47.6 %) and *A/G* (26.7 %)), when compared with the 3rd subgroup (*A/A* – 10,0 %, *A/G* – 23,3 %), the activity of lipoperoxidation processes was significantly higher (DC and MDA - by 35.8 and 30.8 % ($p<0.05$)), the antioxidant system is more reduced (SOD - by 16.3 % ($p<0.05$)), nitrogenous endotoxicosis - more (NO – by 16.2 % ($p<0.05$), the pro-inflammatory activity of the immune system – higher (IL-1β and TNF-α - by 24.2 and 23.4 % ($p<0.05$)), anti-inflammatory system – less (IL-10 – by 17.5 % ($p<0.05$)).

Thus, the obtained results can be argued that in the presence of conditionally "pathogenic" genotypes *G/G* and *A/G* of the *CTLA4* gene, the course of psoriasis is more severe: pronounced oxidative stress, significant inhibition of antioxidant activity, rapid development of nitrogen intoxication, excessive pro-inflammatory response and inhibition anti-inflammatory component of the immune system.

Our data allow us to make another conclusion that in order to select the optimal diagnostic and therapeutic algorithm for psoriasis of varying severity, it is necessary to be guided by the information on genetic analyzes of the *CTLA4* gene polymorphism (*49A/G*). This will make it possible to personalize the approach to the treatment of patients with psoriasis of varying severity, which has recently been given the main role by modern dermatovenerology.

CONCLUSION

1. Patients with psoriasis have excessive activation of lipid peroxidation processes, inhibition of the antioxidant system, development of nitrogenous intoxication and significant disorders of the immune system. In this case, homeostatic disorders are associated with the severity of the pathology, which, in severe degree, are pronounced.
2. In the presence of polymorphic genotypes *G/G* and *A/G* of the *CTLA4* gene, more pronounced and persistent changes in the indices of the homeostasis system are recorded.

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