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SIGNIFICANCE OF THE G-197A POLYMORPHISM OF THE IL17A GENE IN THE FORMATION OF VITILIGO

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Abstract:

Vitiligo is a multifactorial disease, and the destruction of melanocytes develops as a result of genetic mutations, immune and autoimmune reactions, oxidative stress, neuroendocrine changes, the formation of inflammatory mediators, etc.

In recent years, the attention of scientists has been attracted by the participation of a relatively new interleukin-17 (IL-17) in the pathogenesis of some immune-mediated autoimmune diseases, including vitiligo. However, in the literature, the significance of this cytokine is not clearly defined and the data are contradictory. A number of scientific studies have noted an increase in IL-17 in the blood and tissue samples of vitiligo patients compared with control subjects ($p = 0.001$) and positively correlated with the activity and area of the affected body surface. Whereas in other scientific work, the authors were unable to find a correlation between the level of IL-17 and the stage of the disease. To obtain a complete understanding of the studied cytokines, in particular IL-17, it is necessary to investigate the polymorphism of these genes, which will allow predicting the course and determine the effectiveness of therapy depending on the severity of alleles and genotypes of the studied genes in vitiligo patients. In this article, we present the results of a study of the G-197A polymorphism of the IL17A gene in the development of vitiligo in the Uzbek population.

Keywords: Vitiligo, IL17A Gene G-197A Polymorphism, Uzbek Population

Vitiligo is an acquired skin disease characterized by destruction or absence of melanocytes and clinically manifested by well-defined areas of depigmentation of various sizes and shapes and occurs in 0.5-2% of the world's population [1,2,3].

Vitiligo is a multifactorial disease, and melanocyte destruction develops as a result of genetic mutations, immune and autoimmune responses, oxidative stress, neuroendocrine changes, formation of inflammatory mediators and etc[1, 4].

Both humoral and cellular immune system disorders are involved in the pathogenesis of vitiligo. Cytotoxic CD8+ T cells have been found to target melanocytes and are specifically responsible for melanocyte death. The presence of these cells in epidermal and dermal infiltrates was confirmed histologically [5, 6].

In addition, an increased number of CD8+ T-cells was found in the blood of vitiligo patients compared with healthy controls and the findings correlated with vitiligo activity [7, 8, 9].

CD8+ T cells have been found to secrete several cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) [10, 11, 12]. IFN-γ plays a central role in the pathogenesis of vitiligo and promotes the recruitment of autoreactive CD8+ T cells to the skin [13].

Sufficient evidence has accumulated that both proinflammatory (IL-2, IL-6, IL-17, IL-22, and TNF-α) and anti-inflammatory cytokines (IL-10) have been found to play a significant role in the pathophysiology of vitiligo.[14, 15, 16, 17, 18, 19, 20, 21] . It is the imbalance in pro-inflammatory and anti-inflammatory cytokines that may be the trigger mechanism in the occurrence of this pathology.

In recent years, the participation of a relatively new interleukin-17 (IL-17) in the pathogenesis of some immune-mediated autoimmune diseases, including vitiligo, has attracted the attention of scientists. However, the significance of this cytokine is not clearly defined in the literature and the data are contradictory.

A number of scientific studies have reported increased IL-17 in blood and tissue samples from patients with vitiligo compared with control subjects ($p=0.001$) and positively correlated with activity and body surface area affected ($r = 0.0615$, $p<0.001$) [22]. Whereas in another research paper, the authors could not find a correlation between IL-17 levels and disease stage [23].

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To obtain a complete picture of the cytokines studied, particularly IL-17, it is necessary to study the polymorphism of these genes to predict the course and determine the effectiveness of therapy, depending on the expression of the alleles and genotypes of the studied genes in vitiligo patients.

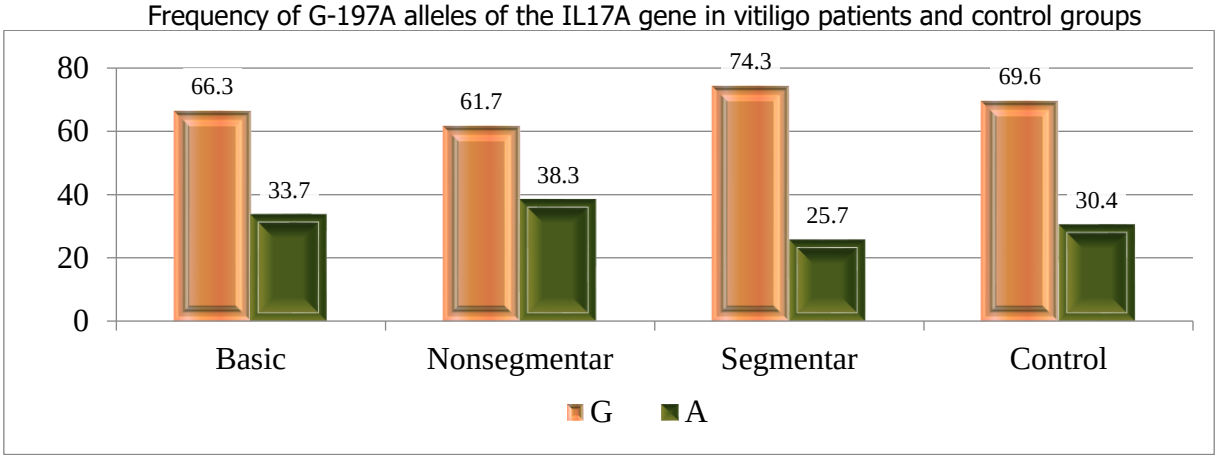
The purpose of this study to test the hypothesis about the possible relationship between polymorphism G-197A gene IL17A with the formation of progressive disorders of skin pigmentation and the role of this polymorphism in the etiopathogenesis of vitiligo and the disease type, we conducted association study in 95 patients with vitiligo (women 47.4% and men - 52.6%) and 92 individuals without dermatosis, conditionally healthy individuals of Uzbek nationality. The results of the studies are shown in Table 1.

The study of G-197A polymorphism of IL17A gene revealed that the G197 allele was dominant in the combined sample of patients with vitiligo and occurred statistically non-significantly lower than in the control group (66.3% vs. 69.6%, respectively; $\chi 2=0.4$; $p=0.5$) (Diagram 1). In contrast, the -197A minor allele was insignificantly more common in vitiligo patients than in controls (33.7% vs. 30.4%, respectively, with $\chi 2=0.4$; and $p=0.5$). The calculated relative chance of finding this allele in patients with vitiligo compared with controls was OR=1.2 with 95%CI:0.75-1.79. Relative risk RR=1.0, at 95%CI:0.67-1.63.

Table 1
Frequency of alleles and genotypes of the G-197A polymorphism of the IL17A gene in vitiligo patients and control groups

N	Group	Allele frequency				Genotype distribution frequency					
		G		A		G/G		G/A		A/A	
		n	%	n	%	n	%	n	%	n	%
1	Main (n=95)	126	66,3	64	33,7	39	41,0	48	50,5	8	8,4
A	Nonsegmental (n=60)	74	61,7	46	38,3	21	35	32	53,3	7	11,7
B	Segmental (n=35)	52	74,3	18	25,7	18	51,4	16	45,7	1	2,9
2	Control (n=92)	128	69,6	56	30,4	43	46,7	42	45,6	7	7,6

Figure 1.
Frequency of G-197A alleles of the IL17A gene in vitiligo patients and control groups



No statistically significant differences were found when comparing the frequency distribution of variant genotypes in the studied groups of vitiligo patients and controls. The dominant genotype of the G-197A polymorphism of the IL17A gene in the patient groups studied was the heterozygous G/A genotype, which occurred in 48/95 (50.5%) patients (Fig. 2). Frequencies of wild-type G/G and unfavorable A/A genotypes were recorded in 39/95 (41.0%) and 8/95 (8.4%) patients, respectively.

Figure 2.
Frequency of genotype distribution of the G-197A polymorphism of the IL17A gene in the groups of patients with vitiligo and controls

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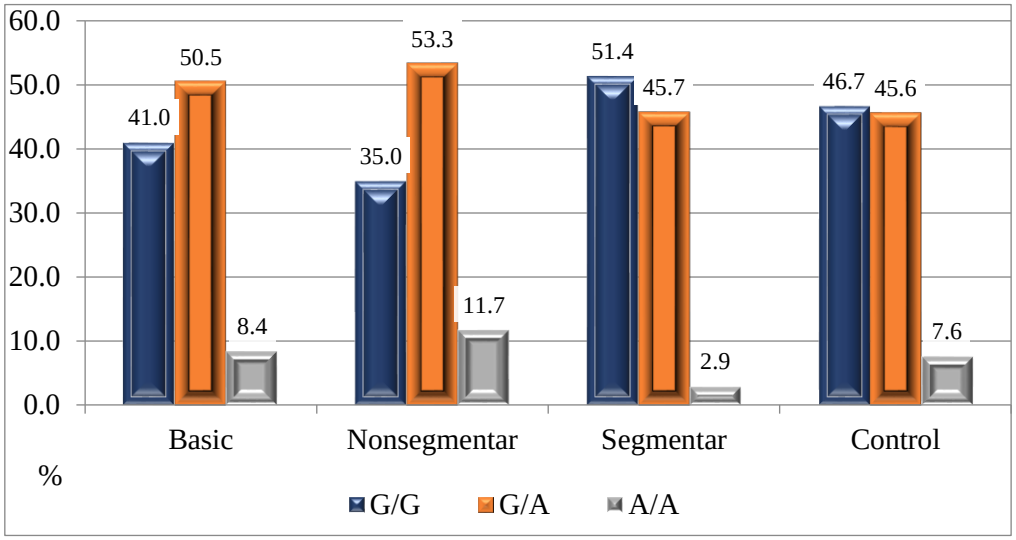

In the group of conditionally healthy donors, the dominant genotype of this locus was the homozygous G/G genotype, which was represented by the frequency of 43/92 (46.7%). The second most common genotype was the heterozygous G/A genotype, which had a frequency of 42/92 (45.6%). The frequency of the minor unfavorable A/ A genotype for this cohort was 7/92 (7.6%). The differences in the frequency of allelic and genotypic variants of the G-197A polymorphism of the IL17A gene in the combined group of patients with vitiligo and the control sample are presented in Table 2.

The proportion of carriage of the ancestral G/G genotype was found to be slightly higher among conditioned donors than vitiligo patients (46.7% vs 41.0%, respectively; $\chi 2=0.6$; $p=0.4$), and this genotype was not associated with a protective effect against vitiligo development (OR=0.9 at 95%CI:0.55-1.33). The proportions of heterozygous genotype in the examined control and patient groups were 50.5% and 45.6%, respectively, ($\chi 2=0.4$; $p=0.5$), and the relative risk ratio was - OR=1.2 at 95%CI:0.68-2.1. The odds ratio of developing vitiligo in carriers of the homozygous A/A genotype was significantly low and was OR=1.1 with 95%CI:0.38-3.21. The relative risk of developing the pathology was also insignificant and was RR=1.1 with 95% CI: 0.41-2.92.

Table 2
Differences in the frequency of allelic and genotypic variants of the G-197A polymorphism of the IL17A gene in the combined group of patients with vitiligo and the control sample

Alleles and genotypes	Number of alleles and genotypes examined				χ^2	p	RR	95%CI	OR	95%CI
	Main group		CG							
	n	%	n	%						
G	126	66.3	128	69.6	0.4	0.5	0.9	0.63-1.43	0.9	0.55-1.33
A	64	33.7	56	30.4	0.4	0.5	1.0	0.67-1.63	1.2	0.75-1.79
G/G	39	41.0	43	46.7	0.6	0.4	0.8	0.49-1.54	0.8	0.44-1.41
G/A	48	50.5	42	45.6	0.4	0.5	1.1	0.63-1.92	1.2	0.68-2.1
A/A	8	8.4	7	7.6	0.04	0.8	1.1	0.41-2.92	1.1	0.38-3.218

Thus, the study of the associative relationship between the G-197A polymorphism of the IL17A gene and susceptibility to the development of vitiligo showed the absence of a clear relationship between this locus and the formation of this pathology. The genotypic variants of this polymorphism were evenly distributed within the studied patient groups and the control sample. The differences detected were not statistically reliable and do not even allow us to identify trends in their distribution ($P>0.05$).

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These data allow us to conclude that the variant polymorphism G-197A of the IL17A gene (associated with high IL17 mRNA expression) does not contribute to the formation of melanogenesis disorder and vitiligo development.

It is known that dividing the combined group into subgroups by shape or other indicators and calculating the frequency of genotypic SNP variants, enhances OR (chance of detection) values and significantly increases the degree of certainty, which allows a more accurate assessment of the level of association with the pathology. Therefore, the next stage of our work was a comparative analysis of the G-197A polymorphism of the IL17A gene in subgroups with nonsegmental vitiligo (NSV) (n=60), and patients with segmental vitiligo (SV) (n=35).

Tables 3, 4 and 5 present the results of comparative analysis of allele and genotype distribution frequencies of IL17A gene G-197A polymorphism in NSV, SV patients and control sample subgroups.

Comparative analysis of the allele and genotype frequencies of the G-197A locus of the IL17A gene between the NSV and SV patient subgroups revealed a tendency for a significant difference in alleles. Therefore, the allele and genotype frequencies of this polymorphism were compared separately for NSV and SV patients with controls (Table 3).

Table 3
Differences in the frequency of allelic and genotypic variants of the G-197A polymorphism of the IL17A gene in vitiligo subgroups the control sample

Alleles and genotypes	umber of alleles and genotypes ned				χ^2	p	RR	95%CI	OR	95%CI
	Subgroup NSV		Subgroup SV							
	n	%	n	%						
G	74	61.7	2	4.3	3,1	0.08	0.8	0.54-1.25	0.6	0.29-1.06
A	46	38.3	18	25.7	3,1	0.08	1.2	0.50-2.87	1.8	0.94-3.42
G/G	21	35.0	18	51.4	2,5	0.1	0.7	0.35-1.32	0.5	0.21-1.18
G/A	32	53.3	16	45.7	0,5	0.5	1.2	0.63-2.13	1.4	0.58-3.12
A/A	7	11.7	1	2.9	2,2	0.1	4.1	0.21-7.51	4.5	0.62-32.3

Comparative analysis of the frequency distribution of variant alleles and genotypes of the G-197A polymorphic locus of the IL17A gene in the subgroup of patients with NSV revealed no obvious statistically significant differences compared to the control sample (Table 4). There was a tendency for the proportion of the ancestral G allele and the wild-type GG genotype to increase in the subgroup of NSV patients compared to controls. The OR value for the G allele was 0.7. The proportion of the ancestral G/G genotype in the studied subgroup of NSV patients and controls was 21/60 (35.0%) and 43/92 46.7%, respectively ($\chi2=2.0$; p=0.2; OR=0.6; 95%CI:0.31-1.19). The insignificantly high frequency of the wild-type genotype in the control group compared to the patient subgroups indicates the lack of a protective (i.e., protective) effect of this genotype with respect to melanocyte destruction and NSV development.

There was a trend toward an increased frequency of the unfavorable minor variant allele 197A among NSV patients compared to the control group (38.3% versus 30.4%, respectively $\chi2=2.0$; p=0.2). Although the probability of developing this form of vitiligo when carrying this variant allele A was greater than >1 (OR=1.4), the lower limit of the 95%CI-adjusted confidence interval values was less than <1 (0.87-2.30)

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Table 4
Differences in the frequency of allelic and genotypic variants of the G-197A polymorphism of the IL17A gene in the nonsegmental vitiligo subgroup and the control group

Alleles and genotypes	Number of alleles and genotypes examined				χ^2	p	R	95%CI	R	95%CI
	Subgroup NSV		CG							
	n	%	n	%						
G	74	61.7	128	69.6	2.0	0.2	0.9	0.51-1.53	0.7	0.43-1.14
A	46	38.3	56	30.4	2.0	0.2	1.1	0.75-1.68	1.4	0.87-2.30
G/G	21	35.0	43	46.7	2.0	0.2	0.7	0.32-1.71	0.6	0.31-1.19
G/A	32	53.3	42	45.6	0.9	0.4	1.2	0.53-2.53	1.4	0.70-2.61
A/A	7	11.7	7	7.6	0.7	0.4	1.5	0.50-4.63	1.6	0.53-4.79

A comparative analysis of the frequencies of variant unfavorable G/A and A/A genotypes in the subgroup of NSV patients also revealed no statistically significant differences compared to the conditionally healthy sample (Table 4).

No statistically significant differences were found in the comparative analysis between the subgroup of patients with SV and the control sample and by the positions of unfavorable genotypes of the G-197A polymorphism of the IL17A gene (Table 5). Interestingly, there was a slight decrease in the frequency of the A/A unfavorable genotype among patients compared to controls (2.9% vs. 7.6%, respectively). However, statistical processing revealed no statistically significant differences ($\chi2=1.0$; $p=0.3$) with very low odds of detection and confidence interval (OR=0.4; 95% CI: 0.04-2.7)

Table 5
Differences in the frequency of allelic and genotypic variants of the G-197A polymorphism of the IL17A gene in the subgroup of segmental vitiligo and the control group

Alleles and wgenotypes	Number of alleles and genotypes examined				χ^2	p	R R	95%CI	R	95%CI
	Subgroup SV		CG							
	n	%	n	%						
G	52	74.3	128	69.6	0.5	0.5	1.1	0.43-2.64	1.3	0.67-2,3
A	18	25.7	56	30.4	0.5	0.5	1.0	0.68-1.27	0.8	0.42-1,47
G/G	18	51.4	43	46.7	0.2	0.6	1.1	0.36-3.32	1.2	0.55-2,63
G/A	16	45.7	42	45.6	0.1	0.9	1.0	0.33-3.03	1.0	0.33-3.03
A/A	1	2.9	7	7.6	1.0	0.3	0.4	0.01-14.2	0.4	0.04-2.7

From the presented data on the assessment of the prognostic efficiency (AUC) of the polymorphism G-197A of the IL17A gene, we can also conclude that the prognostic efficiency of this locus as an independent marker (i.e., in isolation) to assess predisposition to the risk of vitiligo and its various clinical forms is not very high (Table 6). In the pooled group of glaucoma patients, the predictive efficacy of this polymorphism was AUC=0.52 with a high SE=0.7 and low SP=0.3 (OR=1.2; 95%CI: 0.75-1.79; p=0.4).

In the subgroups of patients with NSV and SV, the sensitivity, specificity, and predictive efficiency of this locus were also consistent with SE=0.7, SP=0.3, AUC=0.54, and SE=0.7, SP=0.26, AUC=0.48.

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Table 6.
Prognostic efficiency according to the G-197A polymorphism classifier of the IL17A gene

Groups	SE	SP	AUC	OR	95%CI	p
Basic/control	0,7	0,3	0,52	1,2	0,75-1,79	0,4
Non-segmental/control	0,7	0,38	0,54	1,4	0,87-2,30	0,5
Segmental/control	0,7	0,26	0,48	0,8	0,42-1,47	0,7

Summarizing (and systematizing) our data, we can conclude that the G-197A polymorphism of the IL17A gene is not significantly associated with the formation and clinical course of vitiligo.

Thus, the G-197A polymorphism is associated with a higher level of interleukin IL17A synthesis. High IL17A cytokine concentration in serum is a predisposing factor for increased risk of vitiligo development.

We believe that our results do not contradict the data of the world studies, where was found a significant contribution of polymorphic locus G-197A of IL17A gene in the development of autoimmune pathologies, including some forms of dermatosis. Although vitiligo has a similar pathophysiological basis to these pathologies, it is nevertheless a separate nosological form with its own etiopathogenetic features.

It is important to emphasize that this is one of the few studies on the peculiarities of G-197A polymorphism of IL17A gene in the genetic structure of vitiligo predisposition. To definitively confirm our conclusions and hypothetical assumptions, we believe that an extended study of the entire IL17A gene cluster and other genes synergistically interacting with it is necessary.

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