GENETIC FEATURES OF KIDNEY DAMAGE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Nazarova Nigina Otabek qizi[1*], Jabbarov Azim Atakhanovich[1], Abdukhalikova Nigora Fakhriddinovna[2], Madazimova Dilrukh Khayotzhonovna[1]

[1]Department of Faculty and Hospital Therapy, Tashkent medical academy, Uzbekistan
[2]Department of Normal and Pathological Physiology, Tashkent medical academy, Uzbekistan

Abstract. Systemic lupus erythematosus is an autoimmune systemic disease of the connective tissue, characterized by the formation of many antibodies to its own cells and their components with the development of immune inflammation with damage to many organs and systems. **Purpose of the research.** To assess the prognostic significance of biochemical markers of cytokines for the development of kidney damage in systemic lupus erythematosus. **Materials and methods.** Genetic research methods included: isolation of genomic DNA. For this, venous blood was assessed in a volume of 5 ml and taken into sterile tubes containing EDTA (ethylenediaminetetraacetic acid). Isolation of genomic DNA was carried out by the sorbent method using the reagents of the innuPREP DNA Micro Kit. The DNA concentration was measured using an Eppendorf photometer (Germany). The quality of the obtained DNA samples was assessed on the basis of the optical density of the DNA solution in the region of the protein and nucleic absorption spectra (280 and 260 nm). Statistical data processing was carried out using the Statistica 10 statistical software package. **Results.** The frequency of occurrence of the minor T allele among patients with SLE did not practically differ from that in the control group ($p = 0.08$). The frequencies of the CC, ST and TT genotypes between the studied groups also did not differ ($p = 0.05$, $p = 0.03$ and $p = 0.001$, respectively). Further, the frequency of alleles and genotypes of the C / T polymorphism (-511) of the IL-1β gene was assessed depending on the duration of the disease. Taking into account the distribution of the duration of the course of SLE by percentiles, three groups of patients with the duration of the disease from 0 to 9 years (36.4%, 16 people), from 10 to 21 years (39.4%, 18 people) and from 22 to 50 years (24.2%, 11 people). There were no statistically significant differences in the distribution of genotypes among all patients with SLE ($p = 0.204$, Pearson $\chi 2 = 5.93$). The frequency of occurrence of the minor T allele and the TT genotype did not significantly differ in the groups with SLE duration of 0-9 years, 10-21 years, and 22-50 years. The minor TT genotype was less often detected in patients with SLE duration of 10-21 years, in contrast to patients with SLE duration of 0-9 years ($p = 0.078$), however, the differences found did not reach statistical significance. **Conclusion:** Thus, when studying the polymorphism (-1082) G / A of the IL-10 gene, it was shown that in patients with SLE, the GG genotype was significantly less common, and the minor allele (-1082) A was significantly more frequent, in contrast to the control group.

Key words: gene polymorphism; alleles; systemic lupus erythematosus; inflammatory nephritis; Toll-like receptors; interleukins; genotype.

I. Introduction

Systemic lupus erythematosus is an autoimmune systemic disease of the connective tissue, characterized by the formation of many antibodies to its own

cells and their components with the development of immune inflammation with damage to many organs and systems.

Many factors are involved in the development of systemic lupus erythematosus: genetic predisposition, epigenetic modification, the influence of the external environment (ultraviolet radiation, smoking, infections).

Numerous studies have shown an association between the carriage of certain histocompatibility antigens and systemic lupus erythematosus. Depending on the contribution of genetic factors, 3 subphenotypes are distinguished: subphenotype 1 has HLA-DRB1*0301 (DR3) and lupus nephritis; subphenotype 2 is characterized by a cumulative genetic pattern and the appearance of antibodies (AT) to double-stranded DNA (ds-DNA) with the development of hematological disorders without the formation of ulcers in the oral cavity in young people; in subphenotype 3, candidate genes are absent, skin rash, photosensitization, and neurological disorders are detected [1-8].

At the heart of violations of the mechanisms of innate immunity is the persistent activation of plasmacytic dendritic cells during the interaction of membrane Toll-like receptors with nuclear antigens formed during apoptosis and cell necrosis, which leads to the overproduction of interferon 1, which has the ability to directly or through an increase in the synthesis of a B-lymphocytic stimulator enhance the survival of B cells and the production of autoantigens [9-15].

A special role in the formation of the inflammatory response is played by autoreactive B-lymphocytes, which upon contact with the antigen undergo clonal expansion, which as a result leads to the formation of memory B-cells and plasma cells, and they secrete autoantibodies to many (100) autoantigens, among which antibodies dominate to ds-DNA. Currently, they are the main biochemical markers of systemic lupus erythematosus [16].

An important role in the activation, differentiation, and survival of B cells is played by 2 cytokines belonging to the TNF-α superfamily: BAFF - B-cell activation factor - B-cell activation factor and APRIL - a proliferation-inducing ligand - proliferation-inducing ligand [17].

A significant role in the pathogenesis of systemic lupus erythematosus belongs to proinflammatory (interleukin 1 and 6, TNF-α, interferons of types 1 and 2) and immunoregulatory cytokines (interleukin-10, TGF-β1) [19-21]. Recently, the study of the mechanisms that oppose damage to renal tissue (the system of "self-defense" of the kidney) discusses the disagreement of the action of cytokines in relation to the processes of limiting autoimmune inflammation [22].

II. Purpose of the research

To assess the prognostic significance of biochemical markers of cytokines for the development of kidney damage in systemic lupus erythematosus.

III. Materials and methods

To achieve this goal, the results of studies of 45 patients aged 16 to 76 years were analyzed. Systemic lupus erythematosus was diagnosed according to the American College of Rheumatology. The activity of systemic lupus erythematosus was assessed using the SLEDAI index. All patients gave written consent to participate in the experiment. For comparison, a study of 34 perfectly healthy people was conducted.

Genetic research methods included: isolation of genomic DNA. For this, venous blood was assessed in a volume of 5 ml and was taken into sterile tubes containing EDTA (ethylenediamine tetraacetic acid). Isolation of genomic DNA was carried out by the sorbent method using the reagents of the innuPREP DNA Micro Kit. DNA concentration was measured using an Eppendorf photometer (Germany). The quality of the obtained DNA samples was assessed on the basis of the optical density of the DNA solution in the region of the protein and nucleic absorption spectra (280 and 260 nm).

Statistical data processing was carried out using the Statistica 10 statistical software package.

IV. Results

The frequency of occurrence of genotypes and alleles of the IL-1β gene depending on the presence of systemic lupus erythematosus are shown in Table 1.

Table 1. Distribution of genotypes and alleles of the IL-1β gene polymorphism

Genotype / allele	SLE (n=45), %	Comparison group (n=34), %	p
CC	18,45 (41)	14,4 (42,3)	0,05
CT	20,7 (46)	15,5 (45,6)	0,03
TT	10 (22)	4,1 (12,1)	0,05
CT+TT	26,1 (58)	19,6 (57,7)	0,05
T-allele	35,6 (36)	11,8 (34,9)	0,08

The frequency of occurrence of the minor T allele among patients with SLE did not practically differ from that in the control group ($p = 0.08$). The frequencies of the CC, CT and TT genotypes between the study groups also did not differ ($p = 0.05$, $p = 0.03$ and $p = 0.001$, respectively).

Further, the incidence of alleles and genotypes of the C / T polymorphism (-511) of the IL-1β gene was assessed depending on the duration of the disease. Taking into

account the distribution of the duration of the course of SLE by percentiles, three groups of patients with the duration of the disease from 0 to 9 years (36.4%, 16 people), from 10 to 21 years (39.4%, 18 people) and from 22 to 50 years (24.2%, 11 people).

There were no statistically significant differences in the distribution of genotypes among all patients with SLE (p = 0.204, Pearson $\chi 2$ = 5.93) (Table 2). The frequency of occurrence of the minor allele T and genotype TT did not significantly differ in the groups with SLE duration of 0-9 years, 10-21 years, and 22-50 years. The minor TT genotype was less often detected in patients with SLE duration of 10-21 years, in contrast to patients with SLE duration of 0-9 years (p = 0.078), however, the differences found did not reach statistical significance.

Table 2. Distribution of genotypes and alleles of IL-1β gene polymorphism in patients with systemic lupus erythematosus, depending on the duration of the disease

Genotype	0-9 age, % (n)	10-21 year, % (n)	22-50 age, % (n)
CC	44,4 (6)	46,2 (7)	29,2 (4)
CT	36,1 (5)	48,7 (7)	58,3 (9)
TT	19,4 (3)	5,2 (1)	12,5 (2)
T-аллель	37,5	29,5	41,7

When assessing the presence or absence of renal complications (in the form of inflammatory nephritis) in patients with SLE, depending on the duration of the disease, there were no statistically significant differences in the distribution of genotypes and alleles of the C / T polymorphism (-511) of the IL-1β gene.

Table 3. Distribution of genotypes and alleles of C / T polymorphism (-511) of IL-1β gene in patients with systemic lupus erythematosus, depending on the duration of the disease and the presence of inflammatory nephritis

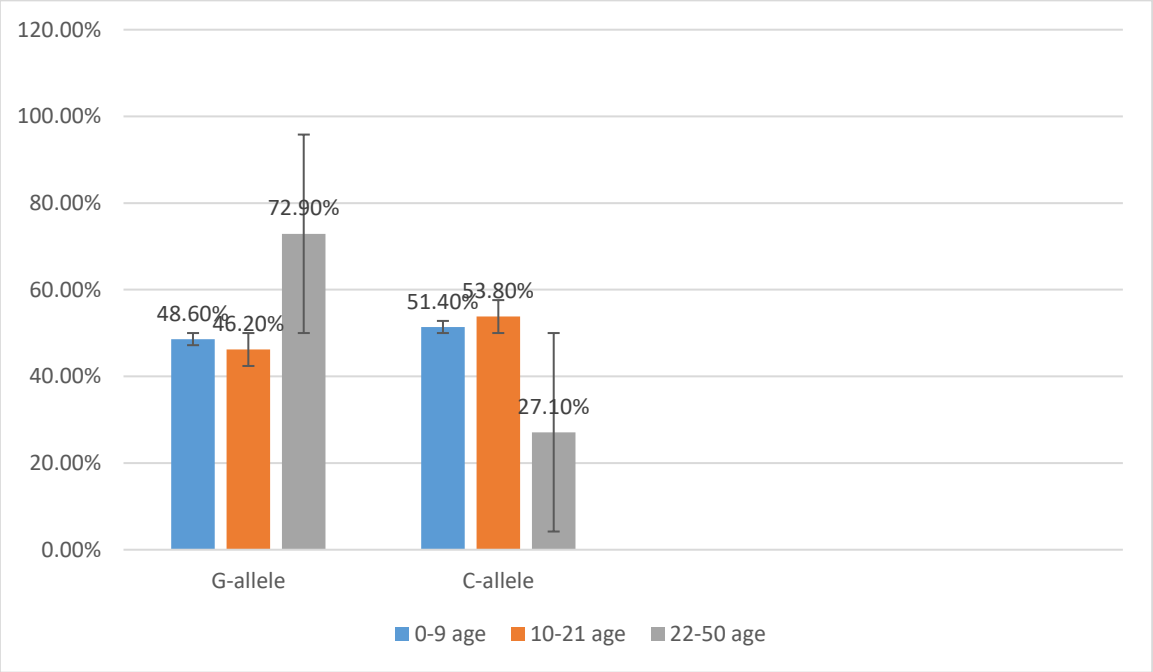
Duration of SLE 0-9 years (p = 0.330, Pearson $\chi 2$ test 2.23)			
Genotype	SLE/IN, % (n)	SLE/GIN, % (n)	p
CC	52,2 (4)	30,7 (2)	0,219
CT	34,8 (3)	38,5 (3)	0,825
TT	13,0 (1)	30,8 (2)	0,208
CT+TT	47,8 (4)	69,3 (5)	0,219
T-allele	30,4 (2)	50 (4)	0,1

Among patients of the subgroup with SLE duration of 0-9 years, the frequency of occurrence of the T allele (-511) and TT genotype was higher in patients without IN than in patients with nephritis (p = 0.430 and p = 0.208, respectively). The same

trend was observed among patients of the subgroup with SLE duration 10-21 years (p = 0.231 and p = 0.438, respectively). However, among patients of the subgroup with a SLE duration of more than 22 years, the allele (-511) T in patients without IN was less common than in patients with nephritis (p = 0.301), and the TT genotype was not found in any patient without IN.

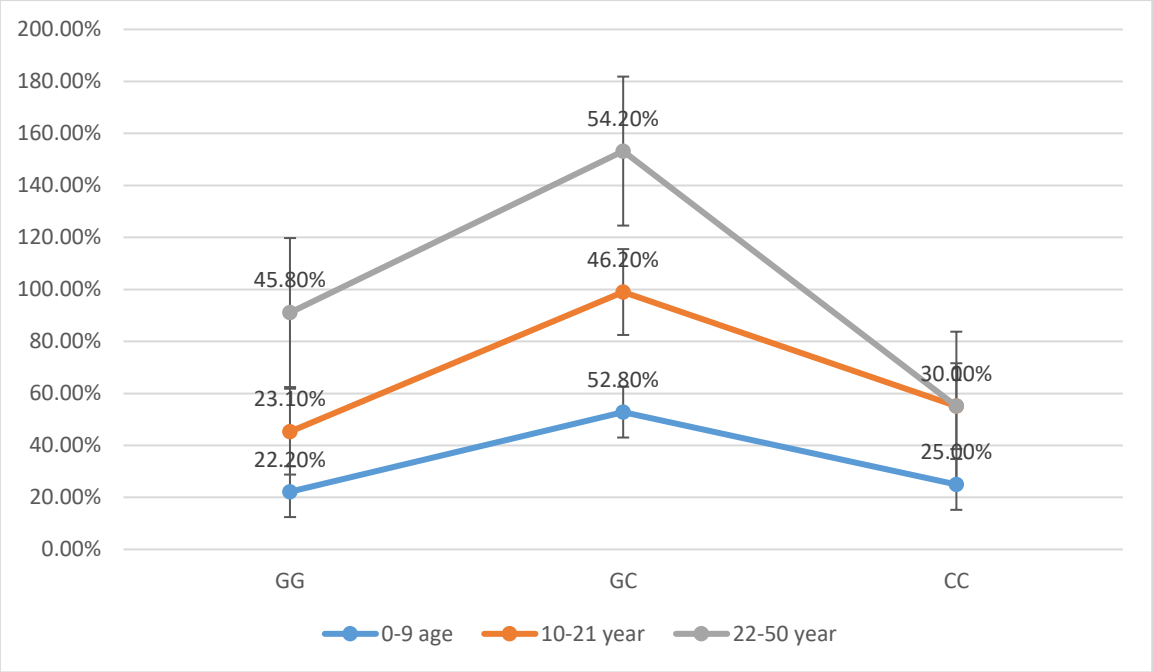
The frequency of occurrence of the minor C allele was 51.4% in the group with SLE duration of 0-9 years and significantly differed from that in the group with SLE duration of 22-50 years (27.1%, p = 0.009). The frequency of occurrence of the minor C allele in the group with SLE duration of 10-21 years was 53.8% and significantly differed from that in the group with SLE duration of 22-50 years (27.1%, p = 0.005) (Fig. 1).

Figure: 1. Distribution structure of alleles of G / C gene IL-6 polymorphism among patients with systemic lupus erythematosus, depending on the duration of the disease



The minor CC genotype was significantly less frequently detected in patients with SLE duration of 22-50 years, in contrast to patients with SLE duration of 0-9 years and 10-21 years (p = 0.008 and p = 0.002, respectively) (Fig. 2).

Figure: 2. The structure of the distribution of genotypes G / C gene IL-6 among patients with systemic lupus erythematosus, depending on the duration of the disease



Thus, in the study of the polymorphism (-174) G / C of the IL-6 gene, no association of this allelic variant with the development of SLE, the presence or absence of VL was revealed. However, when dividing the study sample into subgroups according to the duration of the disease, it was shown that the allele (-174) C and the CC genotype are significantly less common in the subgroup with the longest duration of the disease (22-50 years), in contrast to the subgroups of 0-9 years and 10- 21 years old. In the subgroup of SLE with a duration of 0-9 years, the incidence of the CC genotype was significantly lower among patients with SLE / IN, in contrast to patients with SLE without IN.

The frequency of occurrence of genotypes and alleles of 915G / C polymorphism of the TGF-β1 gene is shown in Table 4. The frequency of occurrence of the minor C allele and the CC genotype among patients with SLE did not practically differ from that in the control group. When assessing the frequency of occurrence of alleles and genotypes among patients with SLE with the presence or absence of IN, statistically significant differences were also not revealed. The frequency of occurrence of the minor C allele and the CC genotype among patients with SLE / GIN was comparable to that in the SLE / IN subgroup.

Table 4. Distribution of genotypes and alleles of 915G / C polymorphism of the TGF-β1 gene

Patients with systemic lupus erythematosus and control group			
Genotype / allele	SLE N=45, %(n)	Control group N=34, % (n)	p
GG	86,3 (39)	86,9 (30)	0,887
GC	13,7 (6)	12,1 (4)	0,702
CC	0 (0)	0 (0)	0,174

GC+CC	13,7 (6)	13,1 (4)	0,887
C-allele	6,9 (3)	7,1 (2)	0,85

The distribution of alleles and genotypes of the 915G / C polymorphism of the TGF-β1 gene among all patients with SLE, depending on the duration of the disease, did not differ statistically significantly ($p = 0.806$, Pearson $\chi 2 = 1.61$) (Table 5). The frequency of occurrence of the minor C allele and the CC genotype was practically the same in patients with SLE duration of 0-9 years, 10-21 years, and 22-50 years. When assessing the presence or absence of VL in patients with SLE, depending on the duration of the disease, no statistically significant differences in the distribution of genotypes and alleles of the 915G / C polymorphism of the TGF-β1 gene were found.

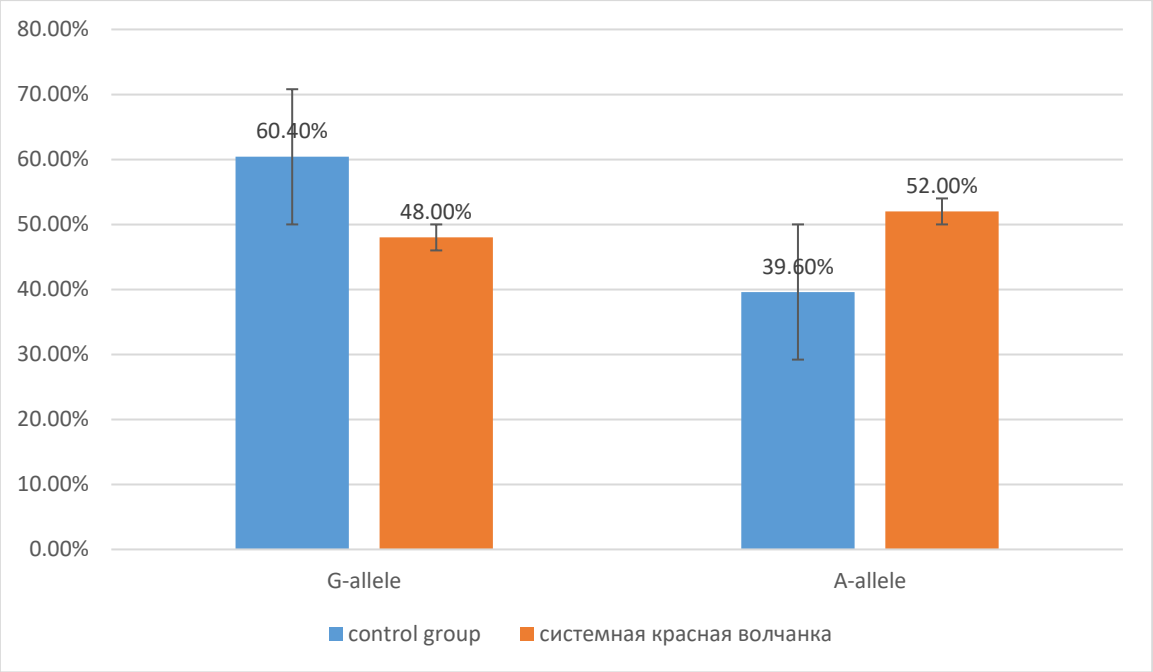
Table 5. Distribution of genotypes and alleles of 915G / C polymorphism of the TGF-β1 gene in patients with SLE, depending on the duration of the disease

Genotype	0-9 age, % (n)	10-21 age, % (n)	22-50 age, % (n)
GG	88,9 (13)	87,2 (13)	87,5 (13)
GC	11,1 (2)	10,3 (2)	12,5 (2)
CC	0 (0)	0 (0)	0 (0)
C-allele	5,5 (1)	7,7 (1)	6,3 (1)

Thus, the study of the 915G / C polymorphism of the TGF-β1 gene did not reveal an association of this allelic variant with the development of SLE, the presence or absence of IN, and the duration of SLE, regardless of the presence or absence of IN in patients.

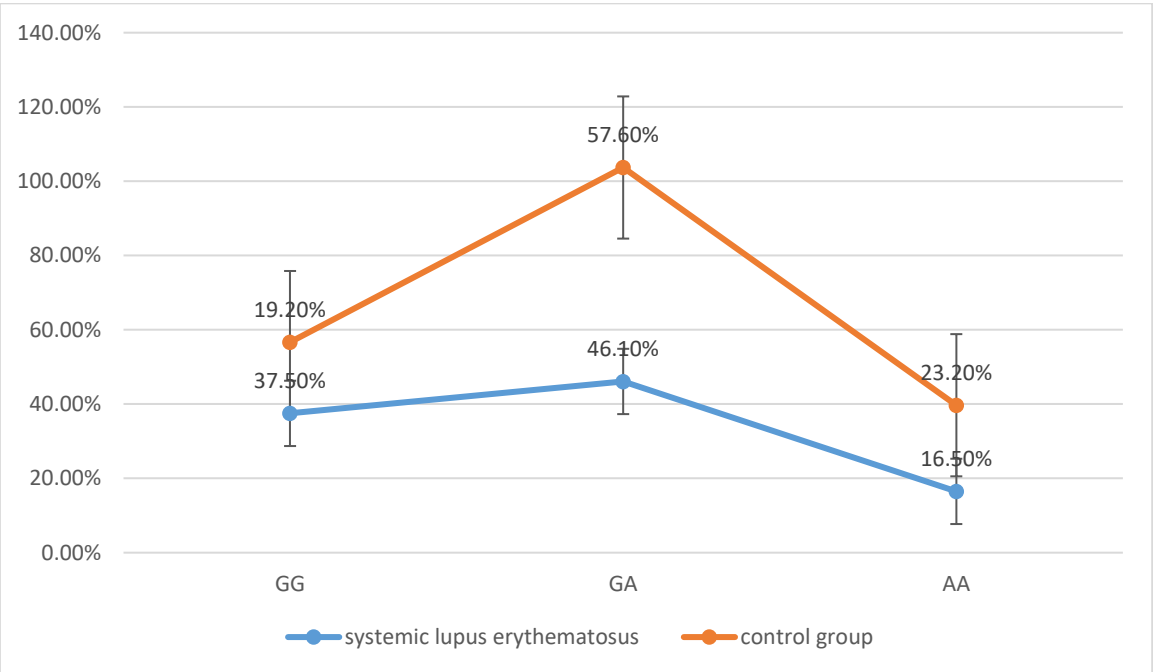
The frequency of occurrence of the minor allele A among patients with SLE was significantly higher than in the control group (Fig. 3).

Figure: 3. Frequency of occurrence of alleles of G / A polymorphism of the IL-10 gene among patients with systemic lupus erythematosus and the control group



The frequency of occurrence of the GG genotype among patients with SLE was significantly lower than in the control group (19.2% and 37.4%, respectively, p = 0.002). At the same time, the carriage of this genotype was associated with a decrease in the risk of developing SLE by almost 2.5 times (p≤0.05). The minor genotype was more common among patients with SLE than in the control group, but these differences did not reach statistical significance (23.2% and 16.5%, respectively, p = 0.202) (Fig. 4).

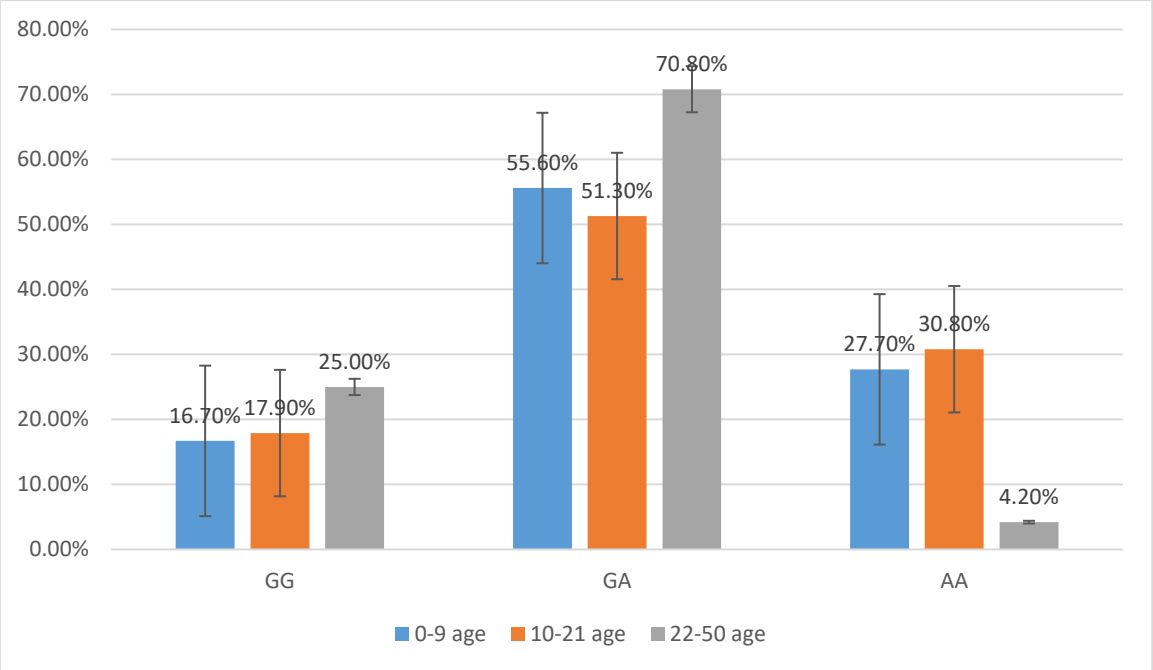
Figure: 4. Frequency of occurrence of genotypes of G / A polymorphism of the IL-10 gene among patients with systemic lupus erythematosus and in the control group



A comparative analysis of the frequency of occurrence of genotypes and alleles in the subgroups of SLE / IN patients and SLE / GIN patients revealed an increase in the frequency of occurrence of the minor allele A among patients with SLE / IN, in contrast to the SLE / GIN subgroup (55.1% and 45.2% acc.), however, these differences did not reach statistical significance (p = 0.221).

There were no statistically significant differences among all patients with SLE in the distribution of genotypes (p = 0.157, Pearson $\chi 2$ = 6.63). The frequency of occurrence of the minor allele A was lower in the group of patients with SLE and disease duration of 22-50 years, in contrast to subgroups with SLE duration of 0-9 years and 10-21 years, but these differences did not reach statistical significance (p = 0.097 and 0.098 acc.). The minor AA genotype was significantly less frequently detected in patients with SLE duration of 22-50 years, in contrast to patients with SLE duration of 0-9 years and 10-21 years (p = 0.037 and p = 0.012, respectively) (Fig. 5).

Figure: 5. Distribution structure of genotypes G / A of the IL-10 gene among patients with systemic lupus erythematosus, depending on the duration of the disease

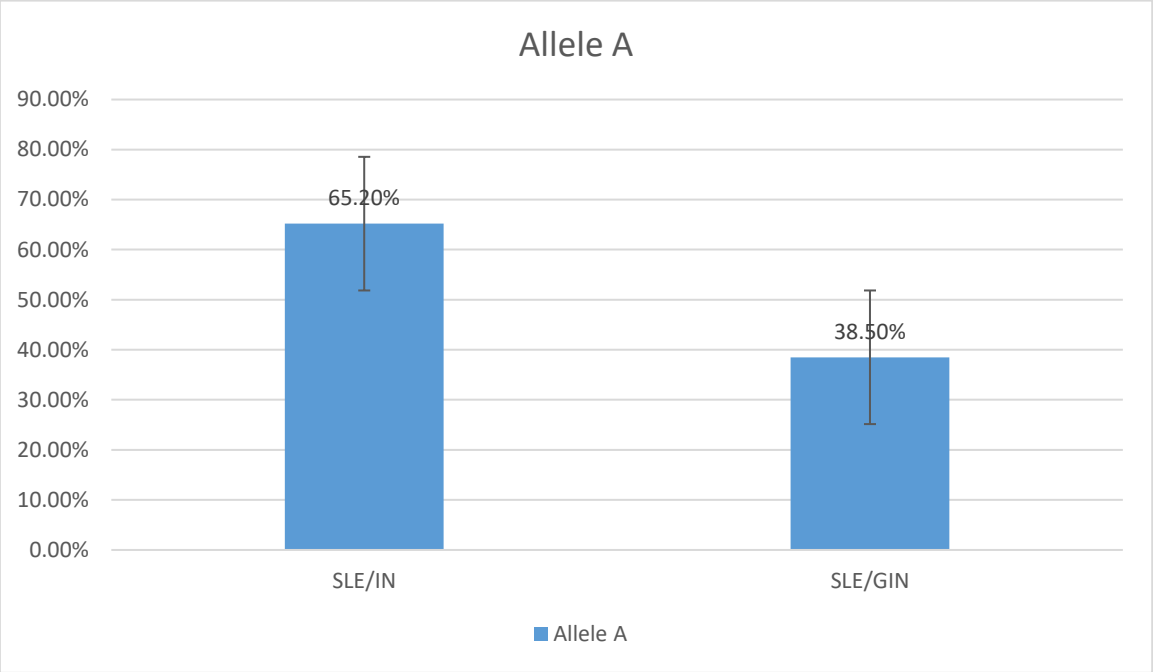


When assessing the presence or absence of IN in patients with SLE, depending on the duration of the disease, no statistically significant differences in the distribution of genotypes and alleles of the (-1082) G / A polymorphism of the IL-10 gene were found.

At the same time, in the subgroup of patients with SLE duration of 0-9 years in patients with IN, the allele (-1082) A was found significantly more often than in

patients without renal complications (65.2% and 38.5%, respectively, p = 0.028) (fig. 6). At the same time, the odds ratio value was 2.99, indicating that the carriage of this genotype increases the risk of developing renal complications almost 3 times in the first 9 years of the disease.

Fig. 6. The frequency of occurrence of the A gene allele in patients with a disease duration of 0-9 years, depending on the presence or absence of inflammatory nephritis



V. Conclusions

Thus, when studying the polymorphism (-1082) G / A of the IL-10 gene, it was shown that in patients with SLE, the GG genotype was significantly less common, and the minor allele (-1082) A was significantly more frequent, in contrast to the control group. The AA genotype was somewhat more common in patients with SLE and significantly more frequent in patients with SLE / IN. The minor AA genotype was significantly less frequently detected in patients with SLE duration of 22-50 years, in contrast to patients with SLE duration of 0-9 years and 10-21 years. Moreover, the (- 1082) A allele was found significantly more frequently in the subgroup of patients with SLE duration of 0-9 years in patients with VL than in patients without renal complications. Therefore, we can assume a negative contribution (-1082) of the A allele and the AA genotype to the development of not only SLE, but also IN. It is noteworthy that this relationship was shown in the subgroup of patients with IN and SLE duration 0-9 years, that is, in the group with the maximum disease activity.

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Authors:

1. Nazarova Nigina Otabek qizi – Assistant of the Department of Faculty and Hospital Therapy, Tashkent medical academy, Tashkent Medical Academy, Farobiy street-2, Tashkent city. 100109, Uzbekistan. *Corresponding author e-mail: niginanazarova33@gmail.com
2. Abdukhalikova Nigora Fakhriddinovna – Researcher of the Department of normal and pathological physiology, Tashkent medical academy, Tashkent Medical Academy, Farobiy street-2, Tashkent city. 100109, Uzbekistan. E-mail: nigora.a@tma.uz
3. Jabbarov Azim Atakhanovich – Professor assistant of the Department of Faculty and Hospital Therapy, Tashkent medical academy, Tashkent Medical Academy, Farobiy street-2, Tashkent city. 100109, Uzbekistan. E-mail: doctor.azim.jabborov@gmail.com
4. Madazimova Dilrukh Khayotzhonovna - Assistant of the Department of Faculty and Hospital Therapy, Tashkent medical academy, Tashkent Medical Academy, Farobiy street-2, Tashkent city. 100109, Uzbekistan. E-mail: madazimovadilruh@gmail.com