International Journal of Psychosocial Rehabilitation, Vol. 24, Special Issue 1, 2020
ISSN: 1475-7192

Analysis of the Col1a1 Collagen Gene in Patients with Limited Scleroderma

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***Abstract*--*Limited scleroderma - a chronic disease of connective tissue with a predominant lesion of the skin and the underlying tissues, characterized by the appearance of sclerosis foci against the background of inflammatory phenomena (erythema, edema) and the subsequent addition of atrophy and hypo / hyperpigmentation of the skin. The set of clinical material was produced at the Republican Specialized Scientific-Practical Medical Center of Dermatovenerology and Aesthetic Medicine. A total of 61 patients with OSD from 4 to 62 years of Uzbek ethnicity were examined. Of these, 22 (36.1 per cent) were men and 39 (63.1 per cent) were women. The control group included 58 practically healthy Uzbek donors from the NIIRPC of the Ministry of Health of Uzbekistan. Molecular genetic studies were carried out in the molecular genetics' laboratory of the NIIRPC of the Ministry of Health. Statistically significant difference was revealed between patients with OSD and healthy donors by the allelic frequencies of the studied polymorphism (rs1107946) of COL1A1 gene ($\chi 2=7.6$; P=0.005; OR=2.3; 95%CI 1.269-4.319). A significant increase in the frequency of heterozygous genotype occurrence in patients with limited scleroderma compared to the control confirms the association of C/A polymorphism genotype (rs1107946) of COL1A1 gene with high risk of OSD formation (OR=2.4). Analysis of dependence of polymorphism distribution of rs1107946 of COL1A1 gene with the course of restricted scleroderma showed that linear form of restricted scleroderma is characteristic for wild (25%) and heterozygous (20%) types, frequency of occurrence of plaque form of disease gradually increases, was revealed in 70; 80 and 100% of patients with wild, heterozygous and mutations. Common foci of limited scleroderma were detected in 14.3; 40 and 60% of patients with the presence of wild, heterozygous and mutation genes. Significant protective effect of functionally favorable genotype A/A polymorphism (rs1107946) of COL1A1 gene for development of restricted scleroderma in Uzbek population is shown, it provokes severe course of disease with appearance of common foci, whereas allele C is prognostically favorable, preventing disease progression.*

Keywords*--*limited scleroderma, collagen, COL1A1 genes, polymorphism

I. INTRODUCTION

One of the manifestations of connective tissue dysplasia is the development of collagen pathology. Among dermatological diseases that accompany the pathology of connective tissue, limited scleroderma (LSD) is most common [1]. Limited scleroderma is a chronic disease of the connective tissue with a primary lesion of the skin and underlying tissues, characterized by the appearance of foci of sclerosis in the background of inflammatory events

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DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020

(erythema, edema) and the subsequent addition of atrophy and hypo / hyperpigmentation of the skin [2]. The disease can occur at any age, characterized mainly by localized foci of chronic inflammation and fibro-atrophic lesions of the skin and mucous membranes [1].

In recent decades, an increase in the number of patients with LSD has been observed. Localized scleroderma is the most common form of scleroderma in children and adolescents, the frequency of the disease is 1:37 000 [3]. In girls, the disease occurs 3-4 times more often than in boys [4]. One of the reasons for the increase in the number of patients with scleroderma in recent years is the change in the body's immunoreactivity, associated with contact with numerous professional and domestic allergens, widespread antibiotic therapy [5].

In the pathogenesis of this pathology, the main importance is attached to the dysfunction of the immune system, metabolic disorders of the components of the connective tissue and microcirculation [2]. The importance of immune disorders in the implementation of scleroderma finds its evidence in a high level of proinflammatory cytokines, the effectiveness of selective immunoactive agents [5]. Defects of humoral and cellular immunity contribute to the formation of autoantibodies, which play an important role in patients with LSD. When LSD observed violations of metabolism of collagen and changes in the vascular endothelium. In all forms of LSD, chronic inflammatory fibrosis of the connective tissue develops, elevated levels of pro-inflammatory cytokines, antibodies to DNA, stimulation of fibroblasts are observed [6].

Transforming growth factor-beta (TGF-β) in the presence of epidermal growth factor (EGF) plays an important role in regulating the profibroz aspects of congenital immune activation [7]. To date, the genetic aspects of the LSD have been little studied in the scientific literature. Molecular genetic studies will allow a deeper understanding of the nature of the formation of collagen in the pathophysiology of scleroderma.

The main genes of the first type of collagen are COL1A1, COL1A2. Along with this, the collagen III-COL3A1 genes are expressed in the dermis; V-COL5A1, COL5A2, COL5A3; VI-COL6A1, COL6A2, COL6A3 [8, 9]. Molecular genetic studies conducted by Ukrainian authors showed the significance of the mutation in the SPINK'5 gene in the development and progression ofLSD, i.e. the presence of allele A in patients causes an increased risk of the occurrence of this pathology, especially in males [10, 11]. According to the authors, this protein plays an important role in various morphophysiological processes in the body, participates in anti-inflammatory and antimicrobial processes, controls the differentiation of epithelium and hair cells, controls the organization of the intercellular matrix, regulates angiogenesis and cell adhesion, etc. [10, 12]. Molecular genetic studies of collagen diseases in persons of Uzbek nationality have not been studied. Finding them out will make it possible to predict the risk of developing LSD, to predict the therapeutic effect of the therapy being conducted.

Objective to study the gene polymorphism of type I collagen in patients with limited scleroderma, depending on the form and course of the disease.

II. MATERIAL AND RESEARCH METHODS

A set of clinical material was produced at the Republican Specialized Scientific and Practical Medical Center for Dermatovenerology and Aesthetic Medicine. A total of 61 patients with LSD aged from 4 to 62 years of

DOI: 10.37200/IJPR/V24SP1/PR201176
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Uzbek nationality were examined. Of these, 22 (36.1%) are men and 39 (63.1%) are women. The control group consisted of 58 practically healthy donors of Uzbek nationality. Molecular genetic studies were performed in the Laboratory of Molecular Genetics of ResearchInstitute Hematology and Blood Transfusion, Ministry of Health of the Republic of Uzbekistan. The analysis of the age qualification of the examined patients showed the following: children of preschool age were 3, school - 7, young - 13, mature - 36 and elderly - 2. The duration of the disease varied widely: from several months to 14 years. Clinical-anamnestic, instrumental, morphological, clinical-laboratory, biochemical, serological and immunological studies were performed on all patients upon admission.

The diagnosis was made on the basis of morphological studies of skin biopsies according to the LSD classification according to the SCLERO.ORG website (this is the world's leading international scleroderma network (InternationalSclerodermaNetwork - ISN) and the International Classification of Diseases X revision, in which the main clinical forms of LSD are presented in class XII "Skin Diseases and subcutaneous tissue ": code L 94.0 - localized (limited) scleroderma, L 94.1 - linear scleroderma, L 94.3 – sclerodactyly.

The clinical classification of LSD, which is based on Highlighted plaque and linear forms of scleroderma. Stage development of foci: erythema, atrophy and sclerosis were established according to the criteria recommended by Acad. V.A.Volnukhin (2013). The criteria for assessing clinical symptoms were the area of the lesion (cm^2), the prevalence of foci: localized from 1-3 foci and common - over 4 foci [2].

Analysis of patients with LSD showed a high incidence of plaque form (Morpheus, Morphea) of scleroderma 53 (86.9%), while the linear (Linear) form was detected in 8 (13.1%) patients. It should be noted that the foci of the plaque form of LSD were localized in 50.9% of cases and widespread in 49.1% of cases. Analysis of the localization of the foci showed that with the linear form of the disease, the foci were mainly localized on the face and, in rare cases, on the body. In patients with a plaque form of the disease, foci were localized on the surface of the limbs in 18 cases, on the face in 22 cases, on the trunk in 31 cases, while on the limbs and trunk they were widespread.

All patients underwent drug treatment, including penicillin, longidase, vasoactive and external drugs. Intramuscular penicillin was prescribed at 1-2 million units per day (course of 20-34 million units); longidase was administered i/m at 32-64 UE every other day (course of 10-15 injections). Patients received intraoral trental- 200-400 mg 2 times a day and / or xanthinolanicotinate - 0.15 g 3 times a day for 1-1.5 months. Topical glucocorticosteroids (Elocom, celestoderm, lorinden) were prescribed from external agents. All patients received 2-3 courses of the indicated therapy with intervalal for 2-3 months.

Clinical symptoms were indexed to assess skin status before and after treatment. The basis of such indexation was the symptom scale proposed by V.A. Volnukhin (2004) - to assess the effectiveness of the treatment of scleroderma. The results of treatment were determined by the number of patients who achieved clinical recovery, significant improvement, improvement of those who did not have effective treatment (lack of effect).

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Serological studies have shown the absence of LE cells in the blood, negative rheumatic tests, and an increase in ASLO was detected. Hematological studies showed no significant differences from the standard values, some increase in the content of monocytes and lymphocytes, as well as an increase in ESR in 68.7% of the patients.

The material for studying the frequency of occurrence of a single nucleotide COL1A1 substitution in the type I collagen gene was genomic DNA samples obtained from peripheral blood leukocytes of patients (61 patients) with LSD (main group), 58 practically healthy individuals (control group) with a set of RNA / DNA extraction from clinical AmpliPrim-RIBO-prep material. To detect the polymorphism of the Type I collagen gene, a polymerase chain reaction (PCR) was performed with a set of reagents for determining the C/A polymorphism of the COL1A1_1 (rs1107946) gene under the guidance of Dr. med. Professor Babayev KT mutations (polymorphism) in the human genome were detected using the “SNP-express” system. Blood samples for PCR were collected in tubes with EDTA "VAC-CUETTE" (Austria). Extraction of genomic DNA from peripheral blood lymphocytes was performed using standard phenol-chloroform deproteinization with some modifications, as well as using the RNA-sorb kits of «InterLabService» LLC and «DNA Express Blood» LLC «Litekh» (Moscow) to determine polymorphism in the human genome COL1A1 in the type I collagen gene according to the manufacturers instructions. The quality of DNA samples was tested on a NanoDrop 2000 “ThermoScientific” (USA) spectrophotometer. Human genomic DNA, isolated from whole blood leukocytes using DNA-express-blood reagent, is analyzed. With the sample of isolated DNA, two amplification reactions are carried out in parallel – with two pairs of allele-specific primers. The results of the analysis allow us to give three types of conclusions: homozygous for allele 1; heterozygote; homozygous for alleles. The SNP-express test systems operate in a narrow range of DNA concentrations: if the DNA concentration is too high, there is a high risk of effectively passing a non-specific reaction and the appearance of false heterozygotes; if the DNA concentration is too low, PCR may fail at all. The working DNA concentration is about 10 ng /μl. Genotyping of the polymorphism of COL1A1_1 (rs1107946) genotypes of the C/A gene was performed using AppliedBiosystems 2720 (USA) programmable thermal cycler using the locus of specific oligonucleotide primers of the sets of the commercial company OOO “Litekh” (Moscow) and OOO “MedLab” (St. Petersburg) manufacturers instructions. Conditions for PCR were optimized by varying the time and temperature parameters of the reaction, as well as using different concentrations of MgCl2 to ensure the specificity of the reaction. The PCR reaction products were analyzed by electrophoresis in 2% agarose gel. DNA fragments were visualized in transmitted ultraviolet light after staining the gel with ethidium bromide at a concentration of 1 μg / ml. The frequency of variants of alleles and genotypes (f) was calculated by the formula: f = n / 2N and f = n / N. Prognostic efficacy (AUC-classifier) of the studied genetic markers was determined by the standard formula: AUC = (Se + Sp) / 2. Correspondence of genotype frequencies to Hardy-Weinberg equilibrium was assessed by the χ^2 criterion. The difference in the frequencies of alleles and genotypes was established using the χ^2 test and the Fisher exact test. Using the software package "OpenEpi 2009, Version 2.3", an assessment of the odds ratio and 95% confidence interval, relative risk assessment (CI and OR) were carried out. The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2013 software package, including the use of built-in statistical processing functions. We used the methods of variation parametric and non-parametric statistics with the calculation of the arithmetic average of the studied

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indicator (M), standard deviation (σ), standard error of the average (m), relative values (frequency,%), the statistical significance of the obtained measurements when comparing the average values were determined by the criterion Student's t (t) with the calculation of the error probability (P) when checking the normality of the distribution (by the criterion of kurtosis) and the equality of the general variances (F is the Fisher criterion). For statistically significant changes took the confidence level of P <0.05. Statistical significance for qualitative quantities was calculated using the χ^2 criterion (chi-square) and z-criterion.

III. RESULTS

One of the main components of the extracellular matrix is collagen. Currently, there are 27 types of collagens, of which 9 types were found in the skin [9]. The most common is type I collagen. When studying the prognostic efficacy of the C / A polymorphism of the gene L1A1_1 (rs1107946), we found a significant increase in the AUC index (P <0.005) in patients with SJD. The predictive efficiency of the rs1107946 polymorphism in this group, according to the AUC values, was 0.63 (P> 0.005), indicating a low prognostic value as an independent marker for predicting the development of LSD (Table 1).

Table 1 Indicators of the predictive efficiency of polymorphism G / A (rs1107946) of the gene COL1A1

N	Genetic markers	SE	SP	AUC	OR (95%CI)	*p
1	rs1107946	0.59	0.67	0.63	2.3	0.005

Note: SE - sensitivity; SP — specificity; AUC - predictive efficiency, * p - Fisher's exact test.

The distribution of alleles and genotypes by C / A polymorphism (rs1107946) of the COL1A1 gene in a population sample and in a group of patients was tested for compliance with the Hardy-Weinberg equilibrium (HWE) using Fisher's exact test. Tables 2 and 3 present the distribution of the expected and observed frequencies of the alleles and genotypes of the rs1107946 COL1A1 polymorphism, as well as indicators of their gene diversity. The distributions of genotypes in the studied samples for a given DNA marker were in equilibrium with HWE, as evidenced by the value of p> 0.05.

Table 2 The expected and observed frequency of distribution of genotypes in the RCS polymorphism of C / A (rs1107946) of the COL1A1 gene in the examined groups

Genotypes	Main group				Control group			
	Genotype frequency		χ^2	P	Genotype frequency		χ^2	P
	Observed	Expected			Observed	Expected		
C/C	0.41	0.45	0,237	0.1	0.67	0.68	0,013	0.5
C/A	0.52	0.44	0,973		0.31	0.3	0,127	

DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020

A/A	0.07	0.11	0,997		0.02	0.03	0,304	
Bcero	1,0	1,0	2,208		1.0	1.0	0,444	

As can be seen from table 2, the C/A polymorphism (rs1107946) of the COL1A1 gene in the studied groups of patients and controls was characterized by a very low frequency of the functionally unfavorable A/A genotype. In the studied group of patients, there was a tendency to a slight increase in the observed frequency of this genotype, compared with the expected values (0.07% vs. 0.11%, respectively). At the same time, the observed distribution of this genotype is consistent with the expected distribution frequencies (P> 0.05). In the population group, despite the distinct differences in the expected and observed frequencies of the unfavorable genotype A/A (0.02 vs. 0.03), statistical analysis showed that such differences are also unreliable (P> 0.05). In the group of patients, the observed frequency of the normal C / C genotype was statistically insignificantly higher than the theoretical value (0.41 versus 0.45, respectively; P> 0.05). In the population group, on the contrary, the observed frequency of this genotype was statistically insignificantly lower compared to the theoretical value (0.67 versus 0.68, respectively; P> 0.05). For the heterozygous genotype C/A, a not very high level of theoretical heterozygosity was detected (from 0.44 to 0.31, respectively). The actual number of the homozygous genotype in the group of patients is not statistically significantly reduced compared with the theoretical (0.52 and 0.44, respectively, χ^2 = 0.973; p> 0.05), and the observed frequency of this genotype in the control group is not significantly higher than the expected (0.31 and 0.3, respectively, χ^2 = 0.127; p> 0.05). The data obtained indicate the homogeneity of the studied sample groups. The results of the analyzes correlate well with each other, which makes it possible to speak about the actual absence of deviations from RCS for a given polymorphism.

At the next stage of the study, the state of the population diversity of the data obtained was estimated by calculating the relative deviation index (D) of the observed heterozygosity from the expected one (Table 3). For each group, the observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}) and H_{obs} deviation index from H_{exp}(D) were calculated. As is known, the heterozygosity of polymorphisms, causes a high viability of organisms, good adaptability to changing environmental conditions. The higher the level of heterozygous state of polymorphisms, the higher their adaptability to the environment. When comparing the differences between theoretical and actual heterozygosity frequencies in the studied group of patients, it was revealed that this marker has a not very high heterozygous deficit index (D = + 0.2) due to an excess of homozygotes (0.41 versus 0.45). Accordingly, it is possible to predict a not very high risk of exposure to the development of LSD in patients of this group. Thus, for a given locus, the indicator D turned out to be negative, i.e. is <0.

However, the level of observed heterozygosity of this polymorphism in the control group was higher than expected (0.31 and 0.3, respectively, D = + 0.03), which suggests we can say equal to the frequencies of the observed heterozygotes, but not theoretically calculated heterozygotes. The frequency of genotypes in the group of conditionally healthy donors also corresponded to RCS (P> 0.05), which allows us to conclude that there are no factors affecting the genetic structure, as well as the correctness of the results of genotyping and the possibility of using the received data.

DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020

Table 3 The difference between the expected and the observed frequencies of the heterozygosity of the polymorphism C / A (rs1107946) of the gene COL1A1

Group	**Observed heterozigosity (H_{obs})**	**Expected heterozigosity(H_{exp})**	**D ***
Main group	0.52	0.44	+0.2
Control group	0.31	0.3	+0.03

The next stage of our work was to study the possible association of allelic and genotypic variants of the rs1107946 polymorphism with the development of LSD. For this purpose, we used the original statistical program OpenEpi (version 9.3). Table 4 presents the results of a comparative analysis of the frequencies of the genotypes of the C / A polymorphism (rs1107946) of the COL1A1 gene between groups of patients with LSD and healthy donors.

Table 4 The frequency distribution of alleles and genotypes of the polymorphism of C / A (rs1107946) of the COL1A1 gene in groups of patients and controls

No	**Group**	**n**	**Allele frequency**				**Genotype distribution frequency**					
			C		**A**		**C/C**		**C/A**		**A/A**	
			n	**%**	**N**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
1	Main group	61	82	67.2	40	32.8	25	41.0	32	52.5	4	6.5
2	Control group	58	96	82.8	20	17.2	39	67.3	18	31.0	1	1.7

When comparing the frequencies of the alleles and genotypes of the C / A polymorphism (rs1107946) of the COL1A1 gene between a general group of patients with LSD and population sampling, statistically significant differences were found. The frequency of C and A alleles was 82 and 40 (67.2 and 32.8%) - in the main group of patients - 96 and 20 (82.8 and 17.2%) - in the control group. As can be seen, a significant increase in the frequency of allele A was detected in the group of patients (32.8%) compared with the control group (17.2%) (χ^2 = 7.6; P = 0.005; OR = 2.3; 95% CI 1.269-4.319). The frequency of the C/C, C/A and A/A genotypes was calculated according to the odds ratio. We found the risk of developing LSD increases almost 2.5 times with the mutant A/A genotype carrier, and this difference was statistically significant (χ^2 = 6.5; P = 0.02; OR = 2.4; 95% CI .159-5.189). These data indicate the association of this polymorphism with the development ofLSD. It is necessary to emphasize that the frequency of the homozygous A/A genotype in the control group, on the contrary, was lower (χ^2 = 1.7; P = 0.2; OR

DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020

= 4; 95% CI 0.434-36.89), and an increase in its occurrence in the main group indicates its protective roles in relation to the development of LSD.

Thus, a significant association was revealed between the “unfavorable” genotypic variant of C/A and the A/A polymorphism (rs1107946) of the COL1A1 gene of the LSD, especially in the subgroup of patients with connective tissue dysplasia. The identification of a functionally unfavorable given genetic marker in patients with various skin lesions makes it possible to predict their risk of forming LSD and determine the further tactics of treatment and preventive measures.

Despite a number of ongoing and quite intensive studies related to the study of the influence of polymorphism (rs1107946) of the COL1A1 gene on the progression of limited scleroderma, data on individual susceptibility to certain skin lesions in patients remain highly controversial. Some scientists have identified a significant association between polymorphism (rs1107946) of the COL1A1 gene with various skin lesions. However, the results of most similar works are contradictory, the opposite results are proved. It should be noted that the progression of the LSD plays a role not only COL1A1, it is possible to have the value of polymorphism and other genes.

The results of the study of the nature of the distribution of allelic and genotypic variants of the rs1107946 polymorphism correspond to patterns obtained in both Caucasoid and Asian populations. An analysis of the distribution of the genotypes of the rs1107946 polymorphism among 58 conditionally healthy Uzbek nationalities and 61 patients diagnosed with LSD revealed the prevalence of homozygous C/C variant (67.2 and 41%, respectively) over heterozygous C/A (31 and 52.5%, respectively) and rare homozygous A/A genotype (1.7 and 6.5%, respectively). Analysis of the dependence of the distribution of the rs1107946 polymorphism of the COL1A1 gene over the course of LSD showed that the wild (25%) and heterozygous (20%) types are characteristic of the linear form, while the presence of mutation is not characteristic of this form. It should be noted that the frequency of occurrence of the plaque form of the disease gradually increases depending on the genotype: if it was detected in 70% of patients with a wild genotype, then with heterozygote it increases to 80%, and in the presence of mutation, to 100%. As can be seen from the above data, allele A is prognostically unfavorable for the individual and contributes to the formation of the plaque form of LSD, especially in the homozygous form.

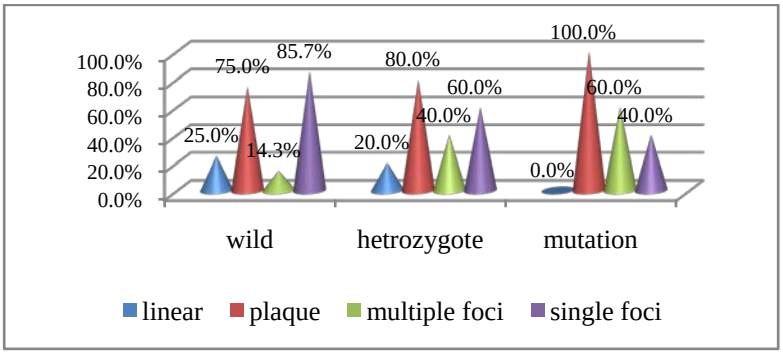

Fig. 1. The relationship of the distribution of the rs1107946 polymorphism of the COL1A1 gene with limited scleroderma.

DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020

Analysis of the course of the disease depending on the rs1107946 polymorphism of the COL1A1 gene showed that the development of multiple LSD foci are detected in 14.3% of patients with the wild-type gene, with heterozygote it increases to 40% and with mutations of this gene is detected in 60% of cases. At the same time, single plaque foci with a high frequency were detected in patients with a wild-type gene (in 85.7% of cases), in the heterozygous form, the frequency decreased to 60%, and in the presence of mutation - to 40%. Based on the data obtained, it can be assumed that the presence of the A allele provokes a severe course of the disease with the appearance of multiple foci, whereas allele C is prognostically favorable, preventing the progression of the disease. The homozygous state - C/C of the studied polymorphic variant was more favorable for their carriers than the heterozygous state - C/A. The protective nature of the homozygous A/A genotype observed by us for the development of LSD can be explained by its association with a low level of gene transcription.

Thus, our results suggest that the polymorphism of the COL1A1 gene is involved in the pathogenesis of LSD. The presence of the C/A genotype of the polymorphism (rs1107946) of the COL1A1 gene in patients can be considered as a genetic predisposition to the development of LSD. In our studies, the likelihood of developing LSD in C/A carriers associated with high expression of COL1A1 was significantly higher (OR = 2.3) than in C/C carriers of the genotype associated with low expression of the COL1A1 protein product. Since allele A of the COL1A1 gene also increases expression of proinflammatory cytokines and is associated with an unfavorable outcome, it is obvious that genotypes with the presence of this allele are among the risk factors for the development of dysplasia. The data obtained open up prospects for the development of new approaches for the early diagnosis of LSD, the identification of risk groups in need of careful follow-up and patient management tactics. It can be said that the definition of this genetic marker in LSD in the Uzbek population has a high probability of the formation of this pathology, however, in our opinion, for the approval of this statement, more large-scale studies with a large number of patients and a clear limitation of the studied population are needed.

IV. CONCLUSION

Analysis of the assessment of the functional significance of polymorphism (rs1107946) of the COL1A1 gene in the development of LSD in patients allowed the following conclusions:

1. A statistically significant difference was found between patients with LSD and healthy donors in the frequencies of allelic variants of the studied polymorphism (rs1107946) of the COL1A1 gene (χ^2 = 7.6; P = 0.005; OR = 2.3; 95% CI 1.269-4.319).
2. A significant increase in the frequency of occurrence of the heterozygous genotype in patients with limited scleroderma compared with the control confirms the C/A genotype of the polymorphism (rs1107946) of the COL1A1 gene to be associated with a high risk of forming LSD (OR = 2.4).
3. Analysis of the dependence of the distribution of the rs1107946 polymorphism of the COL1A1 gene with limited scleroderma showed that the linear form of limited scleroderma characteristic of wild (25%) and heterozygous (20%) types, the incidence of plaque forms of the disease gradually increased in 70, 80 and 100% patients with wild, heterozygous and mutations.

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4. Multiple foci of limited scleroderma were detected in 14.3, 40 and 60% of patients with the presence of the wild, heterozygous gene and the mutation of this gene.
5. A significant protective effect of the functionally favorable A/A genotype of the polymorphism (rs1107946) of the COL1A1 gene was shown for the development of limited scleroderma in the Uzbek population, it provokes a severe course of the disease with the appearance of multiple foci, whereas allele C is prognostically favorable, preventing progression of the disease.

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DOI: 10.37200/IJPR/V24SP1/PR201176
Received: 16 Jan 2020 | Revised: 06 Feb 2020 | Accepted: 25 Feb 2020