World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-41, December 2024
ISSN: 2749-3644

COLLAGEN GENE ANALYSIS IN PATIENTS WITH LOCALIZED SCLERODERMA

Muydinov O.Kh., Khayitov M.S.
Tashkent Medical Academy,
Tashkent, Republic of Uzbekistan

Article history:

Received: September 30th 2024
Accepted: October 28th 2024

Abstract:

Localized Scleroderma (LS) is a chronic connective tissue disorder that predominantly affects the skin and underlying tissues. It is marked by localized areas of sclerosis accompanied by inflammatory symptoms, such as erythema and edema, followed by skin atrophy and alterations in pigmentation (hypo- or hyperpigmentation), as noted by Kubanova in 2010. While LS can occur at any age, it often develops in a localized form, presenting with chronic inflammation and fibroatrophic skin and mucosal lesions. In recent decades, the incidence of LS has increased. Among children and adolescents, it is the most prevalent form of scleroderma, with an estimated prevalence of 1 case per 37,000 individuals. Girls are three to four times more likely than boys to be affected (Kubanova, A.N. Lev., 2013; Kreuter et al., 2006).

Keywords: Localized Scleroderma

Localized Scleroderma (LS) is a chronic connective tissue disorder that predominantly affects the skin and underlying tissues. It is marked by localized areas of sclerosis accompanied by inflammatory symptoms, such as erythema and edema, followed by skin atrophy and alterations in pigmentation (hypo- or hyperpigmentation), as noted by Kubanova in 2010. While LS can occur at any age, it often develops in a localized form, presenting with chronic inflammation and fibroatrophic skin and mucosal lesions. In recent decades, the incidence of LS has increased. Among children and adolescents, it is the most prevalent form of scleroderma, with an estimated prevalence of 1 case per 37,000 individuals. Girls are three to four times more likely than boys to be affected (Kubanova, A.N. Lev., 2013; Kreuter et al., 2006). This rise has been linked to altered immune responses, influenced by exposure to professional and household allergens, as well as increased antibiotic use. **Pathogenesis** The pathogenesis of LS involves significant dysfunctions in the immune system, disruptions in connective tissue metabolism, and microcirculatory irregularities (Kubanova, 2010). Elevated levels of proinflammatory cytokines and the effectiveness of selective immunomodulatory agents highlight the role of immune abnormalities. Both humoral and cellular immune defects facilitate the production of autoantibodies, which are central to LS progression. Key pathological features include altered collagen metabolism, endothelial changes, chronic inflammatory fibrosis, DNA autoantibody formation, and fibroblast activation. Transforming Growth Factor-Beta (TGF-β), along with Epidermal Growth Factor (EGF), plays a pivotal role in regulating profibrotic pathways associated with innate immune activation (Lafyatis and Farina, 2012; Romanova, 2012). However, genetic factors contributing to LS remain largely unexplored. Advances in molecular-genetic studies hold promise for enhancing the understanding of collagen production mechanisms underlying scleroderma. **Key Collagen Genes** Type I collagen production is primarily governed by the COL1A1 and COL1A2 genes. Additional contributions come from genes associated with type III collagen (COL3A1) and types V and VI collagen (COL5A1, COL5A2, COL5A3, COL6A1, COL6A2, and COL6A3), which are highly active in dermal tissues (Torshin, 2007). Ukrainian researchers have identified mutations in the SPINK5 gene as significant in the development and progression of LS. The presence of allele A in affected individuals has been linked to increased disease risk, particularly among males (Savenkova V.V., Zueva M.I.). This protein plays key roles in regulating anti-inflammatory and antimicrobial responses, epithelial cell differentiation, angiogenesis, cell adhesion, matrix organization, and hair cell development (Savenkova et al., 2010). To date, no molecular-genetic studies have been conducted on collagenopathies in individuals of Uzbek descent. Such research could provide valuable insights into predicting LS susceptibility and assessing therapeutic efficacy. **Objective** This study aims to investigate the polymorphism of type I collagen genes in patients with localized scleroderma while taking into account variations in disease form and progression. **Materials

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-41, December 2024
ISSN: 2749-3644

and Methods** Clinical data were obtained from patients at the Republican Specialized Scientific-Practical Medical Center of Dermatovenereology and Aesthetic Medicine. In total, 61 Uzbek patients with LS aged between 4 and 62 years were included, comprising 22 males (36.1%) and 39 females (63.9%). The control group consisted of 58 healthy Uzbek donors. Molecular-genetic analyses were performed at the Molecular Genetics Laboratory of the Research Institute of Gynecology and Perinatology under Uzbekistan's Ministry of Health. The age distribution of LS patients was categorized as follows:

- Preschool-aged children: 3
- School-aged children: 7
- Young adults: 13
- Middle-aged adults: 36
- Elderly adults: 2

The duration of the disease varied widely, ranging from several months to 14 years. Upon admission, all patients underwent comprehensive clinical evaluations that included clinical-anamnestic assessments, instrumental diagnostics, morphological examinations, clinical-laboratory tests, biochemical studies, serological analyses, and immunological investigations.

Analysis of Patients with Limited Scleroderma (LS) The analysis of patients with limited scleroderma (LS) showed a high occurrence of plaque scleroderma, present in 53 patients (86.9%), while the linear form was identified in 8 patients (13.1%). Plaque lesions were solitary in 50.9% of cases and affected multiple areas in 49.1%. Lesion Localization In cases of linear scleroderma, lesions were predominantly located on the face and rarely affected other parts of the body. For plaque scleroderma: - Lesions on extremities were observed in 18 cases, - Facial lesions in 22 cases, - Body lesions in 31 cases. Multiple lesions often appeared simultaneously on the extremities and body. Serological and Hematological Findings Serological observations included: - Absence of LE cells in the bloodstream, - Negative rheumatoid factor tests, - Elevated levels of anti-streptolysin O (ASLO). Hematological results revealed: - Increased monocyte and lymphocyte counts in 68.7% of cases, - Elevated erythrocyte sedimentation rate (ESR), - No substantial deviations from standard values in other parameters. Molecular-Genetic Analysis The study assessed the frequency of a single nucleotide polymorphism in the COL1A1 gene (encoding type I collagen). DNA samples were obtained from the peripheral blood leukocytes of 61 patients with LS (main group) and 58 healthy individuals (control group). Materials and Methods DNA Extraction Genomic DNA was extracted using the AmpliPrim-RIBO-prep kit. This was followed by the phenol-chloroform method with RNA-sorb kits (InterLabService) and DNA Express Blood kits (Litekh, Moscow). Samples were collected in VAC-CUETTE tubes containing EDTA (Austria). Polymorphism Detection Polymerase chain reaction (PCR) was employed with reagents targeting the C/A polymorphism in the COL1A1_1 gene (rs1107946), under the guidance of Professor K.T. Babaev. Mutation analysis was carried out using the SNP-express system. DNA Quality Control DNA sample quality was evaluated with a NanoDrop 2000 spectrophotometer (ThermoScientific, USA), with concentrations adjusted to approximately 10 ng/µL to ensure consistent PCR results. Genotyping and PCR Conditions Genotyping for the COL1A1_1 polymorphism (rs1107946) was performed on the Applied Biosystems 2720 thermal cycler using allele-specific primers from Litekh (Moscow) and MedLab (St. Petersburg). PCR reaction conditions were optimized by fine-tuning temperature, cycles, and $MgCl_2$ concentrations. The obtained PCR products were analyzed using electrophoresis on 2% agarose gel and visualized under UV light after staining with ethidium bromide (1 µg/mL). Genotypic Results Polymorphism analysis identified three genotypic distributions: - Homozygosity for allele 1, - Heterozygosity, - Homozygosity for allele 2. Statistical Analysis Allele and Genotype Frequencies Allele frequencies and genotype distributions were calculated using established formulae and characterized as follows: - Alleles: $f = \frac{n}{2N}$ - Genotypes: $f = \frac{n}{N}$. Predictive efficacy analysis, measured by AUC-classifier values, was computed using $AUC = \frac{Se + Sp}{2}$, where Se represents sensitivity and Sp specificity. Statistical Tests The Hardy-Weinberg equilibrium was examined using the χ^2 test. Differences in allele and genotype frequencies between patient and control groups were assessed with χ^2 and Fisher's exact tests. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using OpenEpi 2.3 software. Software and Parameters Data analysis was conducted using Microsoft Excel-2013, employing integrated statistical functions. Statistical significance was set at $P < 0.05$, with Student's t-test, χ^2, and z-tests applied to validate results. Conclusion This study highlighted critical insights into the genetic basis of limited scleroderma. It established the prevalence of specific lesion types, serological markers, and COL1A1 gene polymorphisms. These findings enhance understanding of disease pathogenesis and offer potential pathways for personalized treatment approaches.

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-41, December 2024
ISSN: 2749-3644

RESULTS. Collagen is one of the key components of the extracellular matrix, with 27 types currently identified, 9 of which are present in the skin. Type I collagen is the most abundant among them. In our study on the prognostic efficacy of the C/A polymorphism in the L1A1_1 gene (rs1107946), we observed a significant increase in the AUC index (P < 0.005) among patients with SD. However, based on the AUC value of 0.63 (P > 0.005), the predictive efficiency of the rs1107946 polymorphism in this group was determined to be low, suggesting it has limited value as an independent marker for forecasting the development of OSD (see Table 1).

Table 1.

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

Distribution of Alleles and Genotypes for the C/A Polymorphism (rs1107946) of the COL1A1 Gene The allele and genotype distribution for the C/A polymorphism (rs1107946) of the COL1A1 gene was analyzed in both the population sample and patient group. Hardy-Weinberg equilibrium (HWE) was assessed using Fisher's exact test. Tables 2 and 3 summarize the observed and expected frequencies of alleles and genotypes for this polymorphism, along with measures of genetic diversity. The genotype distributions for this DNA marker were found to align with HWE, as indicated by p-values greater than 0.05.

Table 2. Observed and Expected Genotype Frequency Distribution for the C/A Polymorphism (rs1107946) of the COL1A1 Gene in the Study Groups

Genotypes	Main group				Control group			
	Frequency of genotypes		χ^2	P	Frequency of genotypes		χ^2	P
	Observed	Expected			Observed	Expected		
C/C	0.41	0.45	0,237		0.67	0.68	0,013	
C/A	0.52	0.44	0,973		0.31	0.3	0,127	
A/A	0.07	0.11	0,997		0.02	0.03	0,304	
Total	1,0	1,0	2,208	0.1	1.0	1.0	0,444	0.5

Table 2 highlights the frequency distribution of the C/A polymorphism (rs1107946) of the COL1A1 gene within the studied patient and control groups. The analysis revealed a remarkably low occurrence of the functionally unfavorable A/A genotype across both groups. In the patient group, there was a slight increase in the frequency of this genotype compared to theoretical expectations (0.07% vs. 0.11%, respectively). However, this difference was statistically insignificant (P > 0.05), indicating that the observed distribution aligns with expected frequencies. In the control group, while a minor discrepancy was noted between observed and expected frequencies of the A/A genotype (0.02 vs. 0.03, respectively), statistical testing confirmed these variations were also not significant (P > 0.05). For the patient group, the observed frequency of the normal C/C genotype was marginally higher than expected (0.41 vs. 0.45, respectively), though not significantly so (P > 0.05). Similarly, in the control group, the observed frequency of the C/C genotype was slightly lower than expected (0.67 vs. 0.68, respectively; P > 0.05). With regard to the heterozygous C/A genotype, theoretical heterozygosity was moderately high, ranging from 0.44 to 0.31. In the patient group, the actual frequency of this genotype was modestly lower than predicted (0.52 vs. 0.44, respectively; $\chi^2 = 0.973$; P > 0.05). Conversely, in the control group, the observed frequency was slightly higher than expected (0.31 vs. 0.3, respectively; $\chi^2 = 0.127$; P > 0.05). These results collectively suggest a high degree of homogeneity within the two sample groups and indicate no substantial deviations from Hardy-Weinberg equilibrium (HWE) for this polymorphism. A broader comparison of allele and genotype frequencies for the C/A polymorphism (rs1107946) between patients with limited scleroderma (LSD) and controls identified significant differences. The C allele was observed at frequencies of 82 (67.2%) in the patient group and 96 (82.8%) in the control group, while the A allele occurred at frequencies of 40 (32.8%) and 20 (17.2%), respectively. The patient group showed a significant increase in the A allele frequency compared to controls ($\chi^2 = 7.6$; P = 0.005; OR = 2.3; 95% CI 1.269–4.319). Analysis of genotype frequencies

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-41, December 2024
ISSN: 2749-3644

(C/C, C/A, and A/A) further underscored this association. The mutant A/A genotype was associated with a nearly 2.5-fold increased risk of developing LSD, a statistically significant finding ($\chi^2 = 6.5$; $P = 0.02$; OR = 2.4; 95% CI 1.159–5.189). These observations imply a strong relationship between this polymorphism and LSD susceptibility. Notably, while the A/A genotype was less frequent in the control group ($\chi^2 = 1.7$; $P = 0.2$; OR = 4; 95% CI 0.434–36.89), its relatively higher frequency in the patient group suggests a potential protective role against LSD development within specific population contexts.

A strong correlation has been observed between the "unfavorable" genotypic variants C/A and A/A of the rs1107946 polymorphism in the COL1A1 gene and the occurrence of limited scleroderma (LSD), particularly in patients with connective tissue dysplasia. Identifying this functionally unfavorable genetic marker among individuals exhibiting various skin lesions provides an opportunity to predict their risk of developing LSD and establish targeted treatment and prevention strategies. Despite extensive research exploring the influence of the rs1107946 polymorphism on LSD progression, findings on individual susceptibility to specific skin lesions remain notably inconsistent. While some studies have identified a significant association between the polymorphism and diverse skin manifestations, a large body of research offers contradictory evidence, often reporting opposing outcomes. It is important to acknowledge that LSD progression may not be solely attributed to COL1A1 variations, as genetic polymorphisms in other loci could also play a substantial role. The distribution patterns of rs1107946 polymorphism genotypes found in this study are consistent with trends reported in both Caucasian and Asian populations. An analysis among 58 healthy Uzbek individuals and 61 LSD patients revealed a higher frequency of the homozygous C/C genotype (67.2% and 41%, respectively) compared to the heterozygous C/A genotype (31% and 52.5%, respectively) and the rare homozygous A/A genotype (1.7% and 6.5%, respectively). Regarding LSD progression, the wild-type (25%) and heterozygous (20%) genotypes were predominantly observed in patients with the linear disease form, while genetic mutations were rarely associated with this subtype. Interestingly, the frequency of the plaque form increased with genotype variation: it occurred in 70% of individuals with the wild-type genotype, rose to 80% in heterozygotes, and reached 100% in those with mutations. These findings suggest that the A allele, especially in the homozygous state, is a prognostically unfavorable factor that predisposes individuals to the plaque form of LSD. A functional analysis of the rs1107946 polymorphism's role in LSD development produced several key insights: - Statistically significant differences were detected in allele frequencies between LSD patients and healthy controls for the rs1107946 polymorphism ($\chi^2 = 7.6$; $P = 0.005$; OR = 2.3; 95% CI: 1.269–4.319). - A prominent increase in the frequency of the heterozygous C/A genotype among LSD patients compared to healthy individuals was observed, linking the genotype to a considerably higher risk of developing LSD (OR = 2.4). - Distribution analyses indicated that the linear form of LSD is predominantly associated with wild-type (25%) and heterozygous (20%) genotypes, while plaque forms appeared with progressively higher prevalence in cases characterized by wild-type (70%), heterozygous (80%), and mutant (100%) genotypes. - The occurrence of multiple foci of LSD was recorded in 14.3%, 40%, and 60% of patients with wild-type, heterozygous, and mutant genotypes, respectively. Importantly, findings demonstrated a protective association of the functionally favorable A/A genotype against LSD development within the Uzbek population. However, paradoxically, this genotype is associated with severe disease progression involving multiple foci. In contrast, the presence of the C allele appears to have a protective effect by mitigating disease advancement.

LITERATURE.

1. Bongers C. , *et al.*
Effectiveness of collagen supplementation on pain scores in healthy individuals with self-reported knee pain: a randomized controlled trial
Appl. Physiol. Nutr. Metabol., 45 (7) (2020), pp. 793-800
2. Chen C.C., Chang S.S., Chang C.H., Hu C.C., Nakao Y, Yong S.M., et al. Randomized, double-blind, four-arm pilot study on the effects of chicken essence and type II collagen hydrolysate on joint, bone, and muscle functions. Nutr J. 2023;22:17.
3. Evans M, Lewis E.D., Zakaria N., Pelipyagina T. , Guthrie N. - A randomized, triple-blind, placebo-controlled, parallel study to evaluate the efficacy of a freshwater marine collagen on skin wrinkles and elasticity - Journal of Cosmetic Dermatology, 20 (3) (2021), pp. 825-834
4. Honvo G., Lengelé L., Charles A., Reginster J.Y. , Bruyère O. - Role of collagen derivatives in osteoarthritis and cartilage repair: A systematic scoping

World Bulletin of Public Health (WBPH)
Available Online at: https://www.scholarexpress.net
Volume-41, December 2024
ISSN: 2749-3644

review with evidence mapping - Rheumatol Ther, 7 (4) (2020), pp. 703-740
5. Khatri M, Naughton RJ, Clifford T, Harper LD, Corr L. The effects of collagen peptide supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise: a systematic review. Amino Acids. 2021;53:1493–506.
6. Miranda de R.B., Weimer P., Rossi R.C. - Effects of hydrolyzed collagen supplementation on skin aging: a systematic review and meta-analysis - Int. J. Dermatol., 60 (12) (2021), pp. 1449-1461
7. Miyashiro R.A., Costa D.H., Deuschle V.C.K.N. - Suplementação de colágeno para envelhecimento cutâneo: Uma revisão - Biomotriz, 16 (1) (2022), pp. 22-35
8. Reilly D.M., Lozano J., - Skin collagen through the lifestages: Importance for skin health and beauty - Plastic and Aesthetic Res, 8 (2021), p. 2
9. Sparavigna A. - Role of the extracellular matrix in skin aging and dedicated treatment - state of the art - Plast Aesthet Res, 7 (2020), p. 14