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Immunogenetic Landscape of Secondary Sjögren's Syndrome in Systemic Sclerosis and Lupus Erythematosus: Insights from Kazakhstan

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Abstract

Introduction: Secondary Sjögren's syndrome (sSS) frequently develops in patients systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). Nevertheless, its immunogenetic features remain poorly understood, particularly in underrepresented populations.

Objective: To investigate the immunological and genetic characteristics of secondary Sjögren's syndrome in Kazakh patients diagnosed with SSc and SLE.

Methods: Patients diagnosed with sSS associated with SLE and SSc were enrolled in the study. SLEDAI-2K and the modified Rodnan skin score were measured, respectively. Antinuclear factor on HEp-2 cells was analyzed by indirect immunofluorescence; autoantibody profiles were determined by immunoblotting. Interleukin (IL)-6 levels were measured by ELISA. Whole-exome sequencing was performed using a targeted panel of autoimmune-related genes. Variants were analyzed and clustered using Ion Reporter software.

Results: Antinuclear factor on Hep2 cells was positive in all SLE-sSS and 75% of SSc-sSS cases. SS-A/Ro60 and SS-A/Ro52 antibodies were frequently detected in both SLE-sSS and SSc-sSS patients, whereas SS-B antibodies were less common. Complement levels were mostly within normal ranges despite 2 SSc-sSS patients. One patient in the SSc group showed an elevated IL-6. Genetic analysis revealed likely pathogenic variants in SAMD9L, ABCC2, IL6ST, and TNFAIP3 in sSS patients.

Conclusion: This study provides insight into the immunogenetic features of secondary Sjögren's syndrome in Kazakh patients, suggesting overlapping genetic patterns in sSS among both SSc and SLE groups. Limitations include the small sample size and cross-sectional design, which limits generalizability, but provide a foundation for further larger-scale research integrating longitudinal follow-up and comprehensive genomic profiling.

Keywords: Secondary Sjögren's Syndrome, Systemic Sclerosis, Systemic Lupus Erythematosus, Antibodies, Genetic Predisposition.

Introduction

Secondary Sjogren's syndrome (sSS) is a chronic autoimmune disease occurs against the background of other autoimmune pathologies, like rheumatoid arthritis (RA), systemic sclerosis (SSc), systemic lupus erythematosus (SLE), polymyositis, and polyarteritis

nodosa [1, 2]. Sjogren's syndrome (SS) characterized by lymphocytic infiltration of the salivary and lacrimal glands, leading to xerostomia and xerophthalmia. Secondary Sjogren's syndrome has a significant impact on the daily activities of patients who often complain about dryness of oral cavity, eyes, as well as dental caries, vaginal dryness, and joint pain [3]. It is accompanied by severe fatigue, depression, anxiety, and decreased physical activity. These conditions can worsen the underlying autoimmune disorder associated with the syndrome and contribute to its more severe clinical manifestations Unfavorable prognostic indicators include delayed diagnosis, male sex, parotid gland enlargement, vasculitis, as well as immunological abnormalities such as hypocomplementemia, cryoglobulinemia, and the presence of anti-SSB antibodies [2].

The etiology of secondary Sjögren's syndrome, like many autoimmune diseases, remains unclear. It is believed that both genetic susceptibility and environmental factors contribute to its development. A variety of exogenous and endogenous elements, including stress, infections, exposure to medications, hormonal fluctuations, and the presence of silicone implants, can disturb immune regulation and initiate the pathological activation of both innate and adaptive immune responses [4]. These pathogens include human T-cell leukemia type 1 virus (HTLV-1), herpes simplex virus (HSV), hepatitis B and C viruses, and cytomegalovirus (CMV) [5]. It is assumed that these viruses are capable of provoking or enhancing autoimmune processes through molecular mimicry, activation of innate immunity, or chronic inflammation. The Epstein–Barr virus (EBV) is considered one of the key infectious triggers of Sjogren's syndrome, as it was found in the tissues of the salivary and lacrimal glands. It promotes the autoimmune process by stimulating epithelial cell apoptosis and activating the innate immune response through Toll-like receptors [6].

Genetic predisposition plays a significant role in the pathogenesis of SS, with both HLA and non-HLA genes being involved in regulating the immune response. HLA-DRB1*0301-DQB1*0201-DQA1*0501 haplotype considered one of the most significant risk factors for the development of SS. One of the most studied genes is interferon-regulating factor 5 (IRF5), which plays a role in the production of type I interferons and the activation of inflammation [7]. A polymorphism in the promoter region of IRF5, specifically the insertion/deletion of the CGGGG sequence, has been associated with increased gene expression and an increased risk of SS [8-10]. One of the first genes found to be associated with the development of SS syndrome was STAT4, this genetic variant is also linked to other autoimmune conditions, such as SLE and RA, highlighting common pathogenic pathways among these disorders [11]. Genetic variants in IL12A, BLK, PTPN22, and CXCR5 show moderate to low association with the development of SS and may be considered as potential risk factors; the same alleles have also been identified in patients with SLE [8, 12]. Disruption in the regulatory pathways involving CHEK1, ETS1, LEF1, TIMP1, and CXCL10 may lead to elevated MMP9 expression, potentially contributing to the development and progression of SS [13].

Autoantibodies play a crucial role in diagnosing Sjögren's syndrome, including its secondary form, which develops in the context of other autoimmune diseases such as SLE and RA. The most characteristic are anti-Ro/SSA and anti-La/SSB antibodies, associated with earlier disease onset, systemic manifestations, and exocrine gland involvement [14, 15]. However, these antibodies are not exclusive to SS and may also be found in other autoimmune conditions. Part of patients may lack these antibodies, highlighting the importance of a comprehensive diagnostic approach that includes clinical evaluation and additional serological markers. In sSS, particularly when combined with RA, rheumatoid factor (RF) and antinuclear antibodies (ANA) are frequently present, complicating differential diagnosis. Emerging markers such as anti-SP1, CA6, NA14 and PSP have been investigated, especially in early stages of the disease [16].

Pathogenesis is initiated by the activation of B- and T-lymphocytes, as well as dendritic cells, which together trigger an autoimmune cascade. T-lymphocytes, especially Th1 and Th17 populations, secrete pro-inflammatory cytokines including interferon-gamma (IFN-gamma) and interleukin-17 (IL-17). IFN-I play role in the pathogenesis of SS by enhancing the activation of immune cells, including NK cells, $CD8^+$ T lymphocytes, and macrophages. Dendritic cells - the main sources of IFN-I, founded in the tissue of the salivary glands of patients, which indicates their possible involvement in the formation of local inflammatory changes [17]. These cytokines contribute to the chronicity of inflammation and damage to exocrine glands. Activated B lymphocytes, among other things by the action of BAFF, differentiate into plasma cells that produce autoantibodies mainly against Ro/SSA and La/SBB antigens, which are found in most patients with SS [4]. Together, these mechanisms contribute significantly to the development and advancement of the disease.

The clinical manifestations of sicca are most often observed in patients with RA (in 31%), SSc (in 20.5%), and SLE (in 8.5%)[18, 19]. In patients with RA, who also suffer from sSS, arthritis is more severe and devastating than in those diagnosed with RA alone [20, 21]. Patients with RA and sSS tend to have higher disease activity by DAS28 [22].

Patients with SLE and sSS exhibit a distinct clinical and serological profile. This subgroup is characterized by a later disease onset, longer disease duration, higher prevalence among women, and a higher risk of chronic disease progression [23]. The most prevalent ocular symptom of SLE is keratoconjunctivitis sicca [24]. Clinically, sSS-SLE is more frequently associated with thyroiditis, arthritis, oral ulcers, interstitial pneumonitis, and renal tubular acidosis. Serologically, these patients demonstrate elevated levels of IgG, rheumatoid factor (RF), and anti-Ro/SSA and anti-La/SSB antibodies [23, 25].

sSS often develops in patients with SS, especially with a limited skin form of the disease. Sicca manifestations are frequently observed in patients with SSc. Among patients with SSc, those with sSS more frequently exhibit anticentromere antibodies and have a lower prevalence of pulmonary fibrosis compared to those without Sjögren's involvement [26]. While histological examination of minor salivary glands in these individuals often reveals significant fibrosis, the presence of lymphocytic infiltration typical for primary Sjögren's syndrome is noted in only a small proportion of biopsies [27].

Secondary Sjögren's syndrome is diagnosed using classification criteria originally developed for primary Sjögren's syndrome (pSS), as no specific criteria for sSS have been officially established. The 2016 ACR/EULAR classification criteria are most commonly applied; they integrate serological, histopathological, and ophthalmological findings. A diagnosis is made when a patient score ≥4 points within this framework and presents with either typical sicca symptoms or systemic activity according to the ESSDAI [28]. Although intended for pSS, these criteria have demonstrated acceptable sensitivity and specificity when applied to sSS [29]. Nonetheless, due to the variable clinical manifestations of sSS, particularly in the presence of coexisting systemic autoimmune diseases, a case-by-case clinical assessment remains essential.

Objective

To investigate the immunological and genetic characteristics of secondary Sjögren's syndrome in Kazakh patients diagnosed with SSc and SLE.

Methods

This cross-sectional study included Kazakh patients diagnosed with SSc (n=30) and SLE (n=30). Patients were recruited from National scientific medical center between July 2023 and July 2024.

Inclusion criteria:

- Confirmed diagnosis of SSc according to the 2013 ACR/EULAR classification criteria; or confirmed diagnosis of SLE according to the 2019 EULAR/ACR classification criteria;
- Diagnosis of secondary Sjögren's syndrome based on the 2016 ACR/EULAR classification criteria;
- Ethnic Kazakh origin.

Exclusion criteria:

- Primary Sjögren's syndrome;
- Other concomitant autoimmune diseases

Control group include individuals without any autoimmune pathology. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Clinical and demographic data were collected from medical records and included age, sex, disease duration, organ injury, and treatment history. SLE patients were assessed for disease activity and impairment of internal organs by SLEDAI-2K score, while for SSc patients we counted modified Rodnan skin score (mRSS).

Whole blood samples were collected for further genetic and immune analysis.

Immunological analysis included ANA HEp2, anti-SSA/Ro, anti-SSB/La, as well as other antibodies common for SLE or SSc, and complement levels (C3, C4). Detection of antinuclear factor on HEp-2 cells (ANA HEp-2) was performed by indirect immunofluorescence in blood serum using an automated fluorescent system (AKLIDES). The presence of specific antinuclear antibodies (dsDNA, Sm, SS-A/60, SS-A/52, SS-B, Scl-70, CENP-B, U1-snRNP, Ribosomal P0, Jo-1, Nucleosome, Histone, RNP/Sm) was investigated using immunoblotting (qualitative result) and indirect immunofluorescence reaction (quantitative result in IU/mL) methods. Levels of C3 and C4 complement components were determined by immunoturbidimetry using a Cobas Integra 400 biochemical analyzer.

Inflammatory cytokine Interleukin-6 was determined by ELISA on an automated immunoassay analyzer Alisei.

Genetic analysis started with DNA Extraction and Library Preparation. Genomic DNA was extracted from whole blood samples using the GENEJET™ Whole Blood Genomic DNA Minikit. DNA quantity was measured with Qubit fluorometry (Qubit 1X dsDNA HS Kit), ensuring sufficient purity and concentration for downstream analysis.

Systematic review of currently available up-to-date literature sources in PubMed and Google Scholar allow to develop the Next-Generation Sequencing panel to identify gene variations relevant to connective tissue disorders, musculoskeletal conditions, and immune-related diseases autoimmune pathology in common, in SLE and SSc particularly [30]. Library preparation involved multiplex amplification using the Ion AmpliSeq Library Kit Plus. Libraries were purified with AMPure XP beads and prepared for emulsion PCR using the Ion PI™ Hi-Q™ OT2 200 system, followed by sequencing on the Ion Proton platform with Ion PI™ v3 chips.

Sequencing data was processed using Ion Reporter software. Initial quality control included filtering short or low-quality reads. High-quality sequences were aligned to a reference genome, and variants (SNPs, indels, structural changes) were identified using the SeqCut tool. Annotations were derived from ClinVar, dbSNP, and other public databases. Filters were applied to prioritize variants: minimum read depth of 30, predicted pathogenic impact (missense, frameshift), and relevance to autoimmune disease. Splice site and gene extension filters were also applied to capture regulatory variants.

Statistical analyses were carried out using Microsoft Excel and IBM SPSS Statistics version 21.0. The choice of statistical tests was guided by the distribution of the data, assessed using the Shapiro–Wilk test for normality. Continuous variables were presented as means ± standard deviation (SD). Hierarchical clustering and heatmap visualization were performed using Ion Reporter Software v5.20 (Thermo Fisher Scientific, USA). Categorical variables were analyzed using Fisher's exact test or the Chi-square (χ^2) test, as appropriate.

Results

Apart from 30 SSc patient twelve individuals were diagnosed with sSS (40%), while within SLE patients it was rare – only 13.3% suffer from sSS (4 patients out of 30 individuals). Descriptive statistics for secondary Sjogren's syndrome are presented in the table 1.

Serological markers

The most common antibodies for sSS according to clinical guidelines and up-to-date research are anti-SS-A/Ro60 and SS-A/Ro52, targeting a protein La (SSB), which plays a role in RNA processing. Due to the data obtained from the current analysis of patients with secondary Sjögren's syndrome, SS-A/Ro60 and SS-A/Ro52 antibodies were frequently detected, while SS-B was less common. Among those with SLE (n = 4), three tested positive for both SS-A/Ro60 and SS-A/Ro52, and one had SS-B antibodies. In the SSc group (n = 12), five patients were positive for SS-A/Ro60, six for SS-A/Ro52, and one for SS-B. This pattern highlights the predominance of SS-A antibodies in both disease contexts. The table 2 summarizes the presence of SS-A/Ro60, SS-A/Ro52, and SS-B autoantibodies in patients diagnosed with secondary Sjögren's syndrome within systemic lupus erythematosus and systemic sclerosis groups. No antibodies were found at the control group.

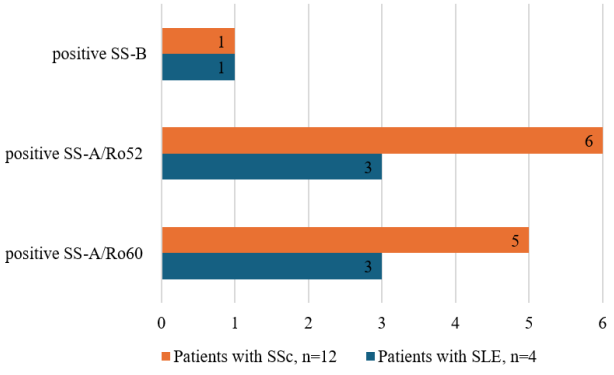

Figure 1 – Autoantibody Profile in Patients with Secondary Sjögren's Syndrome Associated within systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) groups

Table 1 Clinical Characteristics of Patients with SLE and SSc with Secondary Sjögren's Syndrome

No	Sex	Diagnosis	Disease Duration (years)	Steroids (daily dose)	Immunosuppressive Therapy (daily dose)	SLEDAI-2K/ mRSS
L02	**F**	SLE, chronic, active stage 2, with skin and appendage involvement Sjögren syndrome	11	MP 6 mg	Azathioprine 50 mg	22
L13	F	SLE, chronic, with skin and joint involvement, Sjögren syndrome	9	–	Azathioprine 100 mg	6
L14	F	SLE, chronic, with skin (erythema), blood system (anemia), stage 1–2. Sjögren syndrome	19	MP tapering to 8 mg	Hydroxychloroquine 200 mg/day, MMF 1000 mg/day	8
L22	F	SLE, chronic, with multi-organ involvement (skin, vessels, lungs, heart, blood), Sjögren's syndrome	22	MP 6 mg	MMF 1250 mg, Hydroxychloroquine 200 mg	12
S4	F	SSc, chronic, skin, GI tract (esophagitis), Sjögren's syndrome (sialoadenitis, xerostomia)	1	–	MMF 500 mg	2
S6	F	SSc, chronic, stage 2, with skin (edema, induration), joints, lungs (pneumofibrosis, PAH), secondary Sjögren's syndrome	13	MP 8 mg	Nintedanib 200 mg, Leflunomide	9
S11	F	SSc, stage 2, skin, joints, GI (esophagitis), lungs injury (pneumosclerosis), vessels, Sjögren's syndrome	10	MP 4 mg	Leflunomide 20 mg	14
S12	F	SSc, stage 2, skin, joints, vessels (Raynaud's syndrome), lungs (pneumosclerosis), Sjögren's syndrome	7	–	Methotrexate 5 mg/day	9
S15	F	SSc, chronic, with skin edema (face, perioral), Raynaud's, GI, joints, Sjögren's syndrome, Cushingoid, PAH	10	–	Leflunomide 20 mg, D-Penicillamine 500 mg	26
S16	M	Progressive SSc, subacute, stage 2, skin, lungs, vessels, GI, joints impairment, Sjögren's syndrome	3	MP 12 mg	D-Penicillamine 500 mg	15
S18	F	SSc, stage 2, skin, joints, Raynaud's syndrome	15	–	–	19
S19	F	SSc, subacute, stage 2, diffuse, with multisystem involvement, Raynaud's syndrome, Sjögren's syndrome	11	–	Methotrexate 7.5 mg, MMF 1000 mg	6
S21	F	SSc, chronic, stage 1, vascular (Raynaud's syndrome), GI, joints, skin impairment, Sjögren's syndrome	10	–	D-Penicillamine 500 mg	18
S24	F	SSc, chronic, stage 2, limited form, skin, joints, thyroid (AIT), GI injury, Raynaud's syndrome, Sjögren's syndrome	3	MP 8 mg	–	22
S27	F	SSc, generalized, stage 2, skin, joints, GI, lungs, Raynaud syndrome, Sjögren syndrome	12	–	MMF 500 mg	10
S29	F	SSc, chronic, stage 1, skin involvement with sclerodactyly, Raynaud's, GI, joints, lungs impairment	5	–	MMF 1000 mg	20

MP: Methylprednisolone; MMF: Mycophenolate mofetil; GI: Gastrointestinal tract; D-Pen: D-Penicillamine; AIT: Autoimmune thyroiditis; NFS: Functional class; PAH: Pulmonary arterial hypertension; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index; mRSS: Modified Rodnan Skin Score.

Table 2 The frequency of specific antibody to Sjogren's syndrome - anti-SS-A/Ro60 and SS-A/Ro52 in the studied Kazakh cohort of patients diagnosed with secondary Sjögren's syndrome within systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) groups

Secondary Sjögren's Syndrome	positive SS-A/Ro60	positive SS-A/Ro52	positive SS-B
Secondary Sjögren's Syndrome in Patients with SLE, n=4	3	3	1
Secondary Sjögren's Syndrome in Patients with SSc, n=12	5	6	1

In patients with SLE-SS (n=4) with a history of secondary Sjögren's syndrome, other autoantibodies were also detected: dsDNA (1 out of 4), Sm (1 of 4), U1-snRNP (1 of 4), and RNP/Sm (1 of 4). In patients with SSc-SS (n=12), the following additional antibodies were found: Sm (2 of 12), U1-snRNP (4 of 12), RNP/Sm (5 of 12), Ribosomal P0 (2 of 12), and CENP-B (3 of 12). The antinuclear factor on HEp2 cells was positive (at a titer >1:160) in all 4 patients with SLE-SS and the majority of patients with SSc-SS (75%, 9 of 12, Fig. 2). The negative titer <1:80 were found in 3 patients with SSc-SS.

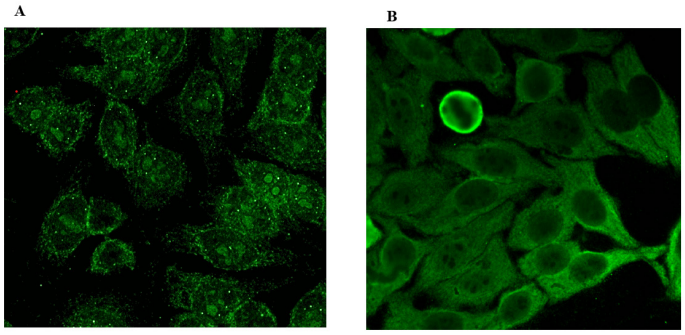

Figure 2 – Antinuclear factor (ANA HEp2). (A) Patient L13, Antinuclear factor on HEp2 cells estimated by IIFA, titre 1:320, nucleolar (AC-8/9/10) and cytoplasmic reticular (AC-21) of glow; positive antibodies to SS-A/52; (B) Patient S06, Antinuclear factor, titre 1:640, nuclear speckled (AC-4/5) and cytoplasmic dense fine speckled (AC-19/20) type of luminescence; positive antibodies to Sm, SS-A/60, U1-snRNP, RNP/Sm, ribP0. *AC – anti-cellular, anapattern.org

Complement and Cytokine Levels in Secondary Sjögren's Syndrome

As shown in Table 3, all patients with secondary SS associated with SLE (n = 4) had C3 and C4 complement levels within the normal reference range. In the SSc-associated sSS group (n = 12), C3 levels were normal in 10 patients and slightly decreased in 2, while C4 was normal in 11 and reduced in 1. Interleukin-6 (IL-6) levels remained within the normal range for all SLE patients, whereas one patient in the SSc group showed an elevated IL-6. Patient S6 (table 1) had skin impairment with edema, induration, joints injury, lungs involvement with pneumofibrosis and pulmonary arterial hypertension (PAH).

Table 3 Levels of C3 and C4 complement components, and interleukin-6 in patients with Secondary S

Indicators	Patients with Secondary SS connected to SLE, n=4			Patients with Secondary SS connected to SSc, n=12		
	normal	below reference interval	above reference interval	normal	below reference interval	above reference interval
Complement C3 (N: 0,90 - 1,80 g/l)	4	0	0	10	2	0
Complement C4 (N: 0,10 - 0,40 g/l)	4	0	0	11	1	0
Cytokine IL-6 (N: 0,0 - 10,0 pg/ml)	4	-	0	11	-	1

Genetic analysis

In patients with Sjögren's syndrome, the variants in the SAMD9L gene - chr7:92764981 T/TT (ref TC) and chr7:92761606 GT/G - were found exclusively in the systemic sclerosis patient group. Two patients, S06 and S15, carried both variants simultaneously. According to the ACMG classification, both variants are categorized as likely pathogenic. The Orphanet database associates the SAMD9L gene with a rare autoinflammatory syndrome characterized by nodular panniculitis, lipoatrophy, severe early-onset interstitial lung disease, and basal ganglia calcification. Most patients exhibit progressive isolated cytopenia affecting B-cells and natural killer (NK) cells.

Notably, two variants in the IL6ST gene were detected in one patient (L22) from the SLE cohort. The variant chr5:55265588 AT/A is classified as likely pathogenic, while chr5:55265655 G/C is of uncertain clinical significance.

Additionally, two likely pathogenic variants in the ABCC2 gene (chr10:101603641 CA/C and chr10:101559041 CA/C) were identified in the systemic sclerosis group. This gene belongs to the transporter protein family, primarily expressed in hepatocytes. While ABCC2 is linked to Dubin-Johnson syndrome (DJS)—an autosomal recessive disorder causing conjugated hyperbilirubinemia—no direct correlation with autoimmune diseases or involvement in immune regulation has been established.

In our study, variants in the IRF5 and STAT4 genes were identified among the patient cohort; however, none of the individuals exhibited clinical features characteristic of Sjögren's syndrome. For example, a variant of uncertain clinical significance in the IRF5 gene (chr7:128588751 AG/A) was detected in two patients with systemic lupus erythematosus and in three individuals diagnosed with rheumatoid arthritis. Additionally, a variant of uncertain clinical significance in the STAT4 gene (chr2:191929615 C/T) was identified in one patient from the rheumatoid arthritis group. It is worth mentioning that in our cohort of patients with systemic sclerosis and Sjögren's syndrome, a likely pathogenic variant in the TNFAIP3 gene (chr6:138199775 T/TC) was also identified.

Other genes included in the panel developed for this study, such as BLK and IKZF1, were not detected in the group of patients with Sjögren's syndrome. However, a variant of uncertain clinical significance in the BLK gene (chr8:11405631 AG/A) was identified in six individuals diagnosed with systemic lupus erythematosus. Additionally, in one SLE patient, a combination of two variants of unknown significance (chr8:11405631 AG/A and chr8:11407753 G/C) was observed.

It was not detected among patients with Sjögren's syndrome, but two variants of uncertain clinical significance in TYK2 (chr19:10476458 C/T and chr19:10472451 C/T) were identified in two individuals diagnosed with rheumatoid arthritis.

Among the participants, two individuals with systemic lupus erythematosus and two with systemic sclerosis were found to carry variants of CTLA4, including likely pathogenic changes at chr2:204732740 GT/G and chr2:204736165 G/GT, as well as a variant of uncertain clinical significance at chr2:204736181 C/T. It is important to note that the chr2:204732740 GT/G variant was also identified in the control group, indicating that no significant statistical association was established between this variant and autoimmune disorders.

The heatmap in the figure 3 provides a visual overview of genetic variants identified in patients with secondary Sjögren's syndrome. Each column represents an individual patient, while each row corresponds to a gene included in the panel. The colors indicate the predicted effect of each variant: darker shades represent more severe impacts (such as splice-site changes), while lighter tones reflect moderate or likely benign effects. Variants of uncertain significance and those with unknown clinical relevance are marked in green and blue. Most patients carried a mix of moderate and low-impact variants, with a few individuals showing high-impact changes that may affect gene function. The presence of recurrent variant patterns across multiple patients suggests potential common pathways involved in immune regulation and connective tissue pathology. This profile underscores the genetic complexity of secondary Sjögren's syndrome and highlights several candidate genes for further functional investigation.

Statistical analysis revealed no significant data in comparison with control group, potentially because of small study group.

Discussion

Our study represents the first immunogenetic investigation of secondary Sjögren's syndrome occurring alongside systemic sclerosis and systemic lupus erythematosus in a Kazakh cohort.

Clinically, the SSc cohort exhibited more extensive organ involvement — pulmonary, vascular, and glandular — while the SLE group tended toward hematologic, mucocutaneous, and glandular involvement. These differences underscore the importance of context-specific diagnosis and tailored

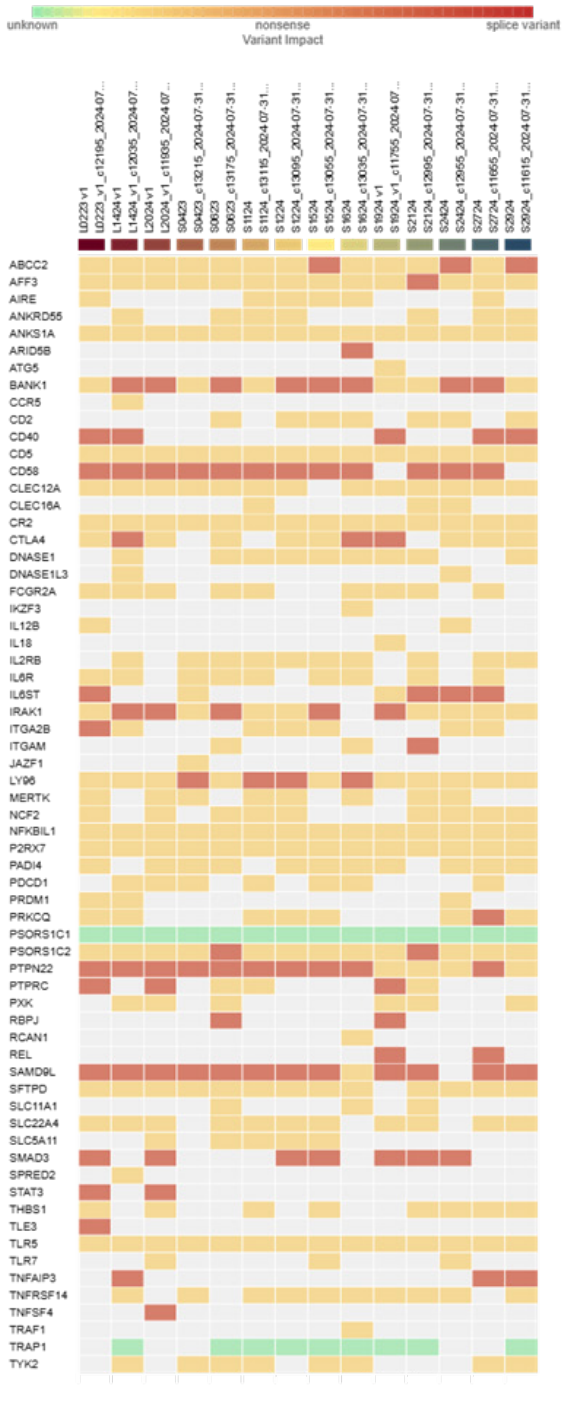

Figure 3 – Heatmap displays the distribution of germline variations across targeted genes in patient samples diagnosed with secondary Sjögren's syndrome (sSS) in the context of systemic sclerosis (SSc) and systemic lupus erythematosus (SLE)

Rows represent the targeted genes, and columns represent individual patient samples. Samples were hierarchically clustered based on the presence and predicted impact of mutations, as determined by Ion Reporter 5.20 (Thermo Fisher Scientific, Darmstadt, Germany). The color scale reflects variant classification: green indicates variants of unknown impact, orange represents missense variants, red denotes nonsense variants, maroon signifies splice variants, and white indicates no detected variant meeting the inclusion criteria. Inclusion criteria for variant reporting were set at a minimum coverage of 30 reads and a variant allele frequency (VAF) threshold of ≥20%. Hierarchical clustering and visualization of the heatmap were conducted utilizing the integrated germline variant annotation tools within Ion Reporter 5.20. Genes are organized according to alphabetical order

therapy when managing sSS depending on its autoimmune background.

We observed that anti SSA/Ro60 and anti SSA/Ro52 antibodies were frequently detected in sSS patients, with Ro52 showing slightly higher prevalence among those with SSc. This aligns with recent studies in SSc identifying anti Ro52 positivity as an independent biomarker for interstitial lung disease (ILD) and progressive lung involvement [31-33]. In fact, a longitudinal study reported that patients with anti Ro52 experienced faster declines in pulmonary function, especially those with early SSc ILD [32]. According to our data 83% of SSc patients with Sjögren's syndrome (5 of 6) had lung impairment in different forms.

Complement protein analysis yielded further insights: all SLE associated sSS patients had normal C3 and C4 levels, while a 2 of SSc associated cases exhibited lower complement values. This may indicate immune complex activation within the SSc group, reflecting differing mechanisms of inflammation in SSc versus the more immune complex–driven pathology typical of SLE.

Interleukin 6 (IL 6) emerged as another finding. The serum level of IL-6 was not elevated in Sjögren's syndrome associated with SLE, however one SSc sSS patient displayed elevated serum IL 6 levels. There was no significant difference in the serum level of IL-6 between groups of the patients with secondary Sjögren's syndrome or control group. The same was found in the literature, nevertheless, numerous recent reports link high IL 6 in SSc to disease severity, presence of digital ulcers, anti Scl 70 antibodies, and cardiopulmonary manifestations such as pulmonary hypertension [34]. Our observation, though limited to a single elevated case, echoes these trends and suggests IL 6 may be a marker of active inflammation and organ involvement in SSc related sSS.

Genetic analysis revealed variants in IL6ST, SAMD9L, CTLA4 and ABCC2 genes. Tala Shahin et al. described patients with inborn errors of immunity and pathogenic IL6ST variants presenting with hyper-IgE syndrome [35]. The strongest association signals are observed in the HLA gene region. In contrast, non-HLA genes such as IRF5 and STAT4 show consistent associations across different ethnic populations, although their effect sizes are comparatively smaller. Most genetic risk variants are located in intergenic regions, and in many cases, their functional roles remain largely unexplored [36]. In another study, in addition to the markers mentioned above, the TYK2 gene SNP rs11085725 was also highlighted [37].

The involvement of additional gene polymorphisms in disease pathogenesis has also been described, with several identified as risk factors. These include polymorphisms in the CD28 cluster of differentiation (CD28 haplotype GC, showing an odds ratio of 2.5 and $P < 0.001$), cytotoxic T-lymphocyte–associated antigen 4 (CTLA4), protein tyrosine phosphatase non-receptor type 22 (PTPN22W), tumor necrosis factor alpha (TNF-α, allele 308 A), interleukin-10 (IL-10, allele 1082 G), and C-X-C chemokine receptor type 5 (CXCR5). Each of these molecules contributes to lymphocyte function at different stages, such as tissue migration (CXCR5), activation and proliferation (CD28, CTLA4), receptor-mediated signaling (PTPN22W), and immunoglobulin secretion (IL-10). For instance, CTLA4 serves as an inhibitory molecule that restrains cell cycle progression and downregulates transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby preventing abnormal T-cell proliferation [38].

Subsequent studies should expand the scope of immunogenetics by utilizing large-scale genome-wide association studies (GWAS) focused on the Kazakh population. Due to the unique ethnicity and genetics of this population, population-based comparative studies would deepen our understanding of the complex genetic factors influencing the risk and disease course of secondary Sjögren's syndrome in the context of SSc and SLE. Clarification of gene-environment relationships, especially with common viral stimuli like Epstein–Barr virus (EBV) and cytomegalovirus (CMV), could aid in formulating tailored precision interventions rooted in genetic data when advanced bioinformatics frameworks are harnessed.

Longitudinal cohort studies are imperative for the characterization of predictive biomarkers for preemptively identifying individuals at increased risk of developing secondary Sjögren's syndrome. The integration of longitudinal clinical phenotyping together with molecular and serological data, including cytokine expression, microRNA profiles, and developing autoantibodies, stands to greatly enhance the prognostic models and early intervention frameworks. In particular, the application of machine learning approaches to diverse datasets has the potential to identify previously uncharacterized clusters of biomarkers.

Prospective, long-term cohort studies are recommended, encompassing detailed clinical evaluations alongside periodic molecular assessments. Incorporating serial measurements of circulating cytokine levels (such as IL-6, IFN-γ, and IL-17), evolving microRNA expression profiles, and autoantibody dynamics (including anti-Ro/SSA and anti-La/SSB antibodies) can significantly enhance predictive accuracy. Implementing advanced bioinformatics approaches, including machine learning algorithms, could facilitate real-time risk stratification, personalized patient management, and target therapeutic interventions aimed at delaying or halting disease progression.

To deepen our understanding of the genetic architecture underlying secondary Sjögren's syndrome, future studies should conduct expansive genome-wide association studies (GWAS) and comprehensive whole-genome sequencing analyses. Emphasis should be placed on recruiting large, ethnically diverse populations, especially considering the distinctive genetic backgrounds present within the Kazakh cohort. Such investigations will likely identify novel susceptibility loci and elucidate the roles of rare variants that may contribute disproportionately to disease pathogenesis. Incorporating population genetics methods, comparative analyses with international genetic databases, and rigorous statistical validation will be instrumental in uncovering critical genetic determinants and previously unexplored pathogenic pathways.

Comprehensive functional analyses are required to substantiate the pathogenic relevance of genetic variants identified in secondary Sjögren's syndrome. In-depth cellular experiments using primary patient-derived immune cells, complemented by CRISPR/Cas9 genome editing technology, will help elucidate variant-specific impacts on cellular signaling pathways, immune cell activation, and cytokine production. Additionally, the generation and analysis of genetically modified animal models will offer indispensable insights into disease mechanisms, organ-specific immune responses, and the efficacy of novel therapeutic interventions. These functional studies hold great promise in advancing personalized treatment strategies and uncovering therapeutic targets tailored specifically to the unique genetic profiles observed in secondary Sjögren's syndrome.

Several notable methodological constraints warrant explicit acknowledgment in the context of interpreting our study findings. Primarily, our investigation included a relatively modest sample size, inherently constraining statistical power and potentially impacting the generalizability and extrapolation of our results to broader patient populations. This limitation increases the possibility of Type II statistical errors, potentially masking genuine associations or exaggerating observed findings. Moreover, the sampling methodology employed may have introduced inadvertent selection biases, particularly given the specific clinical setting and inclusion-exclusion criteria that guided patient recruitment. Such biases could limit the representation of diverse clinical phenotypes and stages of disease progression, thereby narrowing the applicability of findings across different patient subgroups. Additionally, due to practical constraints, the genetic findings in our study were not extensively validated through complementary experimental methodologies, thereby potentially affecting the robustness, reliability, and clinical interpretability of identified genetic variants.

Conclusion

This study offers a first glimpse into the immunogenetic features of secondary Sjögren's syndrome in patients with systemic sclerosis and systemic lupus erythematosus within the Kazakh population. Our findings reveal distinct patterns of autoantibody profiles, complement component levels, cytokine expression, and genetic variants, suggesting both shared and disease-specific immunopathogenic mechanisms. The observed genetic heterogeneity, particularly in immune-related genes, supports the notion of sSS as a complex, polygenic condition influenced by underlying autoimmune disease context. These insights contribute to a more personalized understanding of sSS and may guide future efforts toward targeted diagnostics and therapeutic approaches in ethnically diverse populations.

Author Contributions: L.Z. conceptualized the study, supervised the research process, and contributed to manuscript preparation. A.B. conceived of the presented idea. L.Z. and N.K. were responsible for patient recruitment, D.M. – for clinical data collection, A.B. and Zh.Zh. for sample processing and immune analysis. M.S. and Zh.Zh. performed the genetic analyses and bioinformatic interpretation. L.Z. and L.K. drafted the manuscript, and all authors reviewed and approved the final version.

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