Original Article

Single nucleotide polymorphisms in cytokine genes and their association with primary Sjögren's syndrome in Saudi patients

A cross-sectional study

***Bashaer Alqahtani**, PhD, **Maha Daghestani**, MD, PhD, **Mohammed A. Omair**, MD, **Fahidah Alenzi**, MD, **Esam H. Alhamad**, MD, **Yusra Tashkandy**, PhD, **Nashwa Othman**, PhD, **Arjumand Warsy**, PhD, **Rabih Halwani**, MD.*

ABSTRACT

الأهداف: تحديد التكرارات الأليلية وتأثيرات الاختلافات الوراثية في تعدد أشكال الجينات السيتوكينية في سكان المملكة العربية السعودية.

المنهجية: شملت هذه الدراسة المقطعية 41 مريضا مصابون بمتلازمة سجوجرن الأولية (pSS) و71 مجموعة تحكم صحية بين أكتوبر 2018 ومايو 2019. تم إجراء التنميط الجيني لأشكال النوكليوتيدات الفردية باستخدام نظام SEQUENOM MassARRAY®، الذي يستهدف تسعة أشكال متعددة في جينات السيتوكينات المختلفة. واستخدمت اختبارات مربع كاي لمقارنة المرضى والضوابط.

النتائج: كانت الأنماط الجينية interleukin-1 beta (IL-1β) rs1143627 CT (التحكم، 52.7%؛ المرضى، 21.2%) و TT + CT (p=0.033؛ p=0.003) أقل تواتراً في المرضى الذين يعانون من pSS مقارنة بالضوابط الصحية. ارتبط الأليل C في rs10488631 في جين العامل التنظيمي للفيروسات 5 (IRF5) والأليل A في rs12583006 في جين عامل تنشيط الخلية B (BAFF) بزيادة خطر تطور pSS في مجموعة المرضى.

الخلاصة: لم يرتبط النمط الوراثي CT عند 31− (rs1143627) في IL-1β بارتفاع خطر الإصابة بتطور pSS لدى السكان السعوديين، على عكس ما تم إثباته في الأعراق الأخرى. ومع ذلك، تم ربط الأليل C في rs10488631 في IRF-5 والأليل A في rs12583006 في BAFF.

Objectives: To determine the allelic frequencies and effects of genotypic variations in cytokine gene polymorphisms in a Saudi Arabian population.

Methods: This cross-sectional study involved 41 patients with Primary Sjögren's syndrome (pSS) and 71 healthy controls between October 2018 and May 2019. Single nucleotide polymorphisms genotyping was performed using the SEQUENOM MassARRAY® System, targeting nine polymorphisms in different cytokine genes. Chi-square tests were used to compare the patients and controls.

Results: The interleukin-1 beta (IL-1β) rs1143627 CT (control, 52.7%; patients, 21.2%) and TT + CT (p= 0.003; p=0.033) genotypes were less frequent in patients with pSS than in healthy controls. The C allele in rs10488631 in the interferon regulatory factor 5 (IRF5) gene and the A allele in rs12583006 in the B-cell activating factor (BAFF) gene were associated with an increased risk of pSS development in the patient group.

Conclusion: The CT genotype at −31 (rs1143627) in the IL-1β gene was not associated with a high risk of pSS development in the Saudi population, in contrast to what has been verified in other ethnicities. However, the C allele in rs10488631 in IRF-5 and the A allele in rs12583006 in BAFF were associated.

Keywords: Primary Sjögren's syndrome (pSS), Single nucleotide polymorphism (SNP), Interleukin-1 beta (IL-1β), Interferon regulatory factor 5 (IRF5), B-cell activating factor (BAFF)

Saudi Med J 2023; Vol. 44 (12): 1232-1239
doi: 10.15537/smj.2023.44.12.20230490

From the Department of Zoology (Alqahtani, Daghestani); from the Department of Medicine (Omair), Rheumatology Division; from the Department of Medicine (Alhamad), Pulmonary Division; from the Department of Statistics and Operations Research (Tashkandy), College of Sciences; from the Central Laboratory (Othman); from the Department of Biochemistry (Warsy), King Saud University; from the Department of Clinical Sciences (Alenzi), College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia; and from Department of Clinical Sciences (Halwani), Sharjah Institute for Medical Research, College of Medicine, University of Sharjah, Sharjah, United Arab Emirates.

Received 6th July 2023. Accepted 5th October 2023.

Address correspondence and reprint request to: Dr. Fahidah Alenzi, Department of Clinical Sciences, College of Medicine, Princess Nourah bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia.
E-mail: Fmalenzi@pnu.edu.sa
ORCID ID: https://orcid.org/0000-0001-8495-9064

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by exocrine gland destruction caused by autoreactive lymphocyte infiltration, which results in sicca symptoms.[1] Lymphocyte infiltration affects glandular secretion by altering the glandular structure, leading to cytokine secretion and local autoantibody production.[2]

The etiology of pSS is based on its multifactorial autoimmune nature, whereby several genes influence disease development concurrent with immunological, hormonal, and environmental factors.[3] Cytokines mediate both innate and adaptive immunity. Several studies have confirmed the prevalence of single-nucleotide polymorphisms (SNPs) in cytokine genes that regulate pSS pathogenesis.[4-6]

Several studies involving patients with pSS of different ancestries have identified SNPs in numerous genes implicated in innate immunity, such as interferon regulatory factor 5 (IRF5), signal transducer and activator of transcription 4 (STAT4), and interleukin-(IL) 12.[7-9] However, no studies have described cytokine gene polymorphisms in Saudi Arabian patients with pSS. We hypothesized that cytokine gene polymorphisms are associated with pSS development risk in the Saudi population. In this cross-sectional study, we tested this hypothesis by assessing allelic frequencies and the effect of genotypic variation at different cytokine gene loci in Saudi patients with pSS compared to healthy controls.

Methods. This study is part of a larger clinical study of patients with pSS in Saudi Arabia.[10,11] This cross-sectional study involved 117 male and female Saudi volunteers aged 18–77 years between October 2018 and May 2019. The participants were divided into 2 groups. The first group included 71 healthy subjects with no pSS autoantibodies or symptoms, representing a cross-section of Saudi society. The second group included 46 patients with pSS recruited during routine follow-ups at the rheumatology and pulmonary clinics at King Saud University Medical City in Riyadh, Saudi Arabia. These patients met the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria.[12] The exclusion criteria included a confirmed diagnosis of malignancy, major psychiatric disorder, or the presence of end-stage organ failure. Clinical characteristics, including age, gender, and EULAR Sjögren's syndrome disease activity index (ESSDAI), were assessed during clinic visits. An ESSDAI score of 0 indicated remission, while a score of <5 indicated low disease activity.[13]

The participants provided informed consent, and the study was approved by the Research Ethics Committee of the Medical City at King Saud University. Participants' family histories were recorded. Blood samples were collected from both groups using 3 mL ethylenediaminetetraacetic acid tubes for Deoxyribonucleic acid (DNA) extraction. Deoxyribonucleic acid was extracted using the PureGene blood kit (Gentra Systems, Inc., MN, USA, cat# D5500). The DNA samples were aliquoted and stored at −80°C. It concentration was determined using a nanodrop spectrophotometer (260/280 nm, Thermo Fisher Scientific, Wilmington, DE, USA), and the DNA was diluted to a working concentration of 15–20 ng/µL. Nine SNPs in different cytokine genes with known associations with pSS were selected for further analysis due to the significant effects of cytokine genes in autoimmune diseases. Genotyping was performed using the MassARRAY® System with a complete iPLEX® Pro Genotyping Reagent Set (Lot:0000025499, Agena Bioscience, Inc., Germany). Primers were designed using MassARRAY Assay Design 3.0 (SEQUENOM) and obtained from Aracure (USA). After multiplex PCR amplification using a PCR reagent set (lot:0000025379, Agena Bioscience, Inc.), the final products were desalted and transferred to a SpectroCHIP array (lot:0000025245, Agena Bioscience, Inc.). Allele detection was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Data were analyzed using the MassARRAY TYPER software (version 4.0; SEQUENOM). Duplicate samples, negative controls, and blanks were used to ensure accuracy.

The study was approved by the Institutional Research Board (IRB) of the College of Medicine at King Saud University (E-18-3206). Ethical approval was obtained from the IRB of the College of Medicine at King Saud University (E-18-3206). All procedures were performed in accordance with the Declaration of Helsinki guidelines.

Statistical analyses. Descriptive characteristics were expressed as mean ± standard deviation (SD). A frequency distribution analysis was performed. The genotypes and alleles were assessed for Hardy-Weinberg equilibrium using the Institut für Humangenetik platform.[14] Alle

Disclosure. This study was funded by the Deanship of Scientific Research at King Saud University through the Research Funding Program. The funding body did not have any influence on the study design, study conduct, data analysis, or manuscript preparation.

frequency differences between control subjects and patients were analyzed using the chi-squared test. Relative risk was estimated using odds ratios (ORs) and 95% confidence intervals (CIs). Statistical significance was defined as $p<0.05$. All statistical analyses were performed using Minitab®20 software.

Results. Forty-six patients were recruited; however, only 41 met the inclusion criteria and were included in the final analysis. The mean patient age was 58.76 ± 12.7 years, while in the control group, it was 48.87 ± 14.49 years. There were 32 (78.1%) female pSS patients and 54 (76.1%) female control subjects, while there were 9 (22%) male pSS patients and 17 (24%) male control participants. Please rephrase the word respeticely. The mean disease duration was 4.6 ± 2.28 years (Table 1), and the mean ESSDAI score was 9.95 ± 7.73I. Other clinical characteristics have been previously described.[10] The genotype and allele frequencies of the genes were calculated for each group and compared (Table 2). For all SNPs, the Hardy-Weinberg equilibrium was tested in both groups. The genotype and allele frequencies of several polymorphisms did not differ significantly between the groups: rs16944 in IL-1β, rs1024611 in monocyte chemoattractant protein 1 (MCP-1), rs1234315 in tumor necrosis factor superfamily member 4 (TNFSF4), rs6822844 in IL-2 – IL-21 gene region, rs1800629 in tumor necrosis factor-alpha (TNFβ), and rs7574865 in STAT4. For rs1143627 in the IL-1β gene, the prevalence of the heterozygous (CT) genotype was significantly higher in the control group (52.7%) than in patients with pSS (21.2%). The protective effect of this genotype was evident based on the high OR of 5.524 in the control group. In the IRF5 genes, the rs10488631 C allele frequency was significantly higher in pSS patients (35.9%) and in the BAF genes, the rs12583006 A allele frequency was significantly higher in pSS patients I (40%) than in the control group in which the rs10488631 C allele frequency was (11.4%) and the rs12583006 A allele frequency was (25.8%).

Table 1 - Demographic variables and outcome measures.

Variable	Patients with pSS (n=41) (mean ± SD)	Control (n=71) (mean ± SD)
Age	58.76 ± 12.7	48.87* ± 14.49
ESSDAI	9.95 ± 7.73	-
Disease duration (years)	4.6 ± 2.28	-

ESSDAI: EULAR Sjögren's syndrome disease activity index, *$p<0.001$. pSS: Primary Sjögren's syndrome, SD: standard deviation

Discussion. Various genetic factors contribute to pSS.[15-17] In this study, we assessed 9 SNPs in cytokine genes to determine their association with pSS predisposition or resistance.

Our study demonstrated that 57.3% of healthy Saudi individuals had the minor allele (T) frequency of IL-1β rs1143627. This was similar to the data obtained from multiple populations according to the 1,000 Genome Project, including the Han Chinese in Beijing (54.4%), Colombians (53.7%), Southern Han Chinese (53.8%), and Japanese in Tokyo (52.9%).[18] The prevalence of the heterozygous CT genotype was significantly lower in patients than in healthy controls.

Interleukin-1β encodes IL-1β, a potent pro-inflammatory cytokine that acts as an important inflammatory response mediator and is involved in many important cellular functions.[19] The rs1143627 SNP is a polymorphic site in the promoter region of IL-1β.[20] The C > T transition occurs at position −31 from the transcription start site and lies within the TATA box, thus influencing the formation of the transcription initiation complex and induction of IL-1β expression.[21,22]

Several studies have reported an association between rs1143627 at the IL-1β gene and autoimmune disorders in various populations (Table 3). One study reported that the TT genotype frequency was lower in Japanese patients with pSS than in control subjects ($p=0.027$). Consequently, the mutant T allele has been reported to be protective.[23] The frequency of the T allele was 19.6% in patients compared with 29.2% in controls.[23] Our results are consistent with those of a previous study and show that this mutation has a protective effect against pSS. Other studies have reported several strong associations between the minor allele of rs1143627 and rheumatoid arthritis and immune thrombocytopenia in Indian populations, systemic lupus erythematosus (SLE) in an Egyptian population, and Alzheimer's disease in a Chinese population.[24-27] In contrast, the CT genotype is associated with an increased risk of systemic sclerosis in Caucasians.[28] However, the homozygous CC genotype and C allele have demonstrated a protective effect in Iranian patients.[29] In a population of Chinese children, the AA genotype of rs1143627 was associated with an increased risk of coronary artery lesions in patients with Kawasaki disease.[30]

The risk of gastric cancer has been associated with the T haplotype in Asian populations.[21] In contrast, the opposite haplotype (such as the C allele) is the risk-related allele in Caucasians.[31] The minor T allele has also been associated with cervical cancer in Chinese Uygur

Table 2 - Supplemental genotype and allele frequencies of cytokine gene polymorphisms in patients with primary Sjögren syndrome (pSS) versus controls

Gene	Variant	Genotype/allele	Patients with pSS n (%)	Control group n (%)	OR (95% CI)	X^2	*P*-value
Interleukin-1 beta (IL-1β)	rs16944			Genotype frequency (pSS=33; HC=60)			
		AA	5 (15.2)	13 (21.7)	Reference		
		AG	12 (36.4)	19 (31.7)	0.609 (0.173–2.146)	0.596	0.440
		GG	16 (48.5)	28 (46.7)	0.673 (0.203–2.236)	0.418	0.518
				Allele frequency			
		A	22 (33.33)	45 (37.5)	Reference		
		G	44 (66.7)	75 (62.5)	0.833 (0.443–1.567)	0.320	0.571
	rs1143627			Genotype frequency (pSS=33; HC=55)			
		CC	12 (36.4)	9 (16.4)	Reference		
		CT	7 (21.2)	29 (52.7)	5.524 (1.672–18.250)	7.856	0.005*
		TT	14 (42.4)	17 (30.9)	1.619 (0.530–4.946)	0.715	0.398
				Allele frequency			
		C	31 (47.0)	47 (42.7)	Reference		
		T	35 (53.0)	63 (57.3)	1.187 (0.643–2.193)	0.301	0.583
Interferon regulatory factor 5(IRF5)	rs10488631			Genotype frequency (pSS=32; HC=57)			
		TT	18 (56.3)	44 (77.2)	Reference		
		TC	5 (15.6)	13 (22.8)	1.064 (0.331–3.421)	0.011	0.918
		CC	9 (28.1)	0 (0.0)	-	-	-
				Allele frequency			
		T	41 (64.1)	101 (88.6)	Reference		
		C	23 (35.9)	13 (11.4)	0.229 (0.106–0.496)	14.009	<0.001*
Monocyte chemoattractant protein 1 (MCP-1)	rs1024611			Genotype frequency (pSS=29; HC=65)			
		AA	19 (65.5)	35 (53.9)	Reference		
		AG	8 (27.6)	16 (24.6)	1.086 (0.393–2.999)	0.025	0.874
		GG	2 (6.9)	14 (21.5)	3.800 (0.780–18.511)	2.731	0.098
				Allele frequency			
		A	46 (79.3)	86 (66.3)	Reference		
		G	12 (20.7)	44 (33.9)	1.961 (0.943–4.077)	3.254	0.071
B-cell activating factor (BAFF)	rs12583006			Genotype frequency (pSS=35; HC=64)			
		TT	14 (40.0)	37 (57.8)	Reference		
		TA	14 (40.0)	21 (32.8)	0.568 (0.228–1.416)	1.475	0.225
		AA	7 (20.0)	6 (9.4)	0.324 (0.093–1.134)	3.108	0.078
				Allele frequency			
		T	42 (60.0)	95 (74.2)	Reference		
		A	28 (40.0)	33 (25.8)	0.521 (0.280–0.969)	4.235	0.040*
Tumor necrosis factor Superfamily Member 4 (TNFSF4)	rs1234315			Genotype frequency (pSS=32; HC=59)			
		CC	12 (37.5)	13 (22.0)	Reference		
		CT	12 (37.5)	24 (40.7)	1.846 (0.648–5.259)	1.318	0.251
		TT	8 (25.0)	22 (37.3)	2.538 (0.822–7.836)	2.624	0.105
				Allele frequency			
		C	36 (56.3)	50 (42.4)	Reference		
		T	28 (43.6)	68 (57.6)	1.749 (0.946–3.232)	3.180	0.075
Interleukin-2 – interleukin-21 gene region (IL-2 – IL-21)	rs6822844			Genotype frequency (pSS=32; HC=58)			
		GG	28 (87.5)	49 (84.5)	Reference		
		GT	3 (9.4)	9 (15.5)	1.653 (0.412–6.627)	0.503	0.478
		TT	1 (3.1)	0 (0.0)	-	-	-
				Allele frequency			
		G	59 (92.2)	107 (92.2)	Reference		
		T	5 (7.8)	9 (7.8)	0.993 (0.318–3.099)	0.000	0.990

*$p<0.05$, CI: confidence interval, OR: odd ration, HC: healthy control

women.[32] Moreover, an association between genetic polymorphisms in the IL-1 family and hepatocellular carcinoma has been reported.[33] The heterozygous genotype has been shown to be protective against prostate cancer in a Turkish population.[34] Asian patients with the CC genotype have a significantly increased risk of breast cancer.[35] Several clinical conditions have been associated with rs1143627.

Table 2 - Supplemental genotype and allele frequencies of cytokine gene polymorphisms in patients with primary Sjögren syndrome (pSS) versus controls (continuation).

Gene	Variant	Genotype/ allele	Patients with pSS n (%)	Control group n (%)	OR (95% CI)	X^2	*P*-value
Tumor necrosis factor-alpha (TNFβ)	rs1800629	Genotype frequency (pSS=26; HC=56)					
		GG	18 (69.2)	41 (73.2)	Reference		
		GA	6 (23.1)	8 (14.3)	0.585 (0.177–1.933)	0.772	0.380
		AA	2 (7.7)	7 (12.5)	1.537 (0.290–8.133)	0.255	0.613
		Allele frequency					
		G	42 (80.8)	90 (80.4)	Reference		
		A	10 (19.2)	22 (19.6)	1.027 (0.447–2.360)	0.004	0.951
Signal transducer and activator of transcription 4 (STAT4)	rs7574865	Genotype frequency (pSS = 26; HC = 59)					
		TT	1 (3.9)	8 (13.6)	Reference		
		TG	10 (38.5)	21 (35.6)	0.263 (0.029–2.395)	1.406	0.236
		GG	15 (57.7)	30 (50.9)	0.250 (0.029–2.188)	1.569	0.210
		Allele frequency					
		T	12 (23.1)	37 (31.4)	Reference		
		G	40 (76.9)	81 (68.8)	0.657 (0.309–1.395)	1.197	0.274

*$p<0.05$, CI: confidence interval, OR: odd ratio, HC: healthy control

Table 3 - Association of rs1143627 in the interleukin-1 beta (IL-1β) gene with different autoimmune diseases in populations of different ancestries.

Ref.	Disease	No. (patients/ controls)	Associated genotype or allele	OR (95% CI)	*P*-value	Frequency (patient vs. control)	Ethnicity
[23]	Primary Sjögren syndrome (pSS)	101/106	TT	0.59 (0.31–1.13)	0.027	19.6 vs. 29.2	Japanese
[24]	Rheumatoid arthritis	180/249	TT	1.21 (0.92–1.61)	0.01	0.26 vs. 0.15	Indian
[25]	Systemic lupus erythematosus	50/25	TT	8.27 (1.53–44.61)	<0.01	26 vs. 8	Egyptian
[26]	Alzheimer's disease	123/120	TT	1.72 (1.13–2.61)	0.010	51 (41.5) vs. 31 (25.8)	Chinese
[27]	Immune thrombocytopenia	118/100	TT CT T	2.33 (1.069–5.09) 2.044 (1.068–39) 1.52 (1.04–2.22)	0.033 0.034 0.034	25.43 vs. 19 55.08 vs. 47 52.69 vs. 42.5	Indian
[28]	Systemic sclerosis	78/692	TC	2.7 (1.5–5.1)	0.002	62.0 vs. 43.8	Caucasian
[29]	Systemic lupus erythematosus	163/180	CC CC	0.6 (0.4–0.9) 0.7 (0.5–0.9)	<0.01	52 (32) vs. 81 (45) 191 (58.6) vs. 247 (68.6)	Iranian
[30]	Coronary artery lesions in Kawasaki disease	719/1401	AA	2.28 (1.32–3.95)	0.003	50 vs. 29	Chinese

CI: confidence interval, OR: odd ratio,l vs: versus, ref: reference, no.: number

In the current study, the control group's T allele frequency of rs10488631 at IRF5 was (88.6%), while the control group's TT genotype frequency of rs10488631 at IRF5 was (77.2 %). This is similar to frequencies seen from other populations, such as the British population in England and Scotland (89.6%), populations of North and Western European ancestries (88.9%), Utah residents with Northern and Western European ancestry (88.9%), a Finnish population in Finland (88.4%), and Sri Lankan Tamilians from the United Kingdom (UK) (87.6%).[18]

In this study, there was an association between the C allele of rs10488631 at IRF5 and an increased risk of pSS development in the patient group. These results are consistent with those of Nordmark et al,[36] who reported that the C allele in rs10488631 is a risk allele in Norwegian and Swedish patients with pSS.

In rs10488631, the T > C transition occurs 5 kb downstream of IRF5 near the transportin 3 (TNPO3) gene.[36] Because of its location at the 3′ end, it is difficult to distinguish whether the association signal from this SNP with pSS originates from IRF5 or TNPO3, and no clear functional role can be predicted for this SNP. However, in a genome-wide association study of pSS, researchers found an association between SNPs in the IRF5-TNPO3 gene region and DNA methylation,

indicating that the risk allele can affect DNA methylation levels.[37] High levels of IRF5 might increase the risk of autoimmune development by promoting apoptosis and activating Toll-like receptors and autoantigens.[38]

Several studies on different ethnic groups have reported an association between the IRF5 gene variant (rs10488631) and multiple autoimmune diseases, such as SLE in Swedish, Caucasian, Amerindian, and Greek populations, systemic sclerosis in Caucasian and Turkish populations, lupus in Egyptian populations, and scleroderma in European populations.[38-46]

In the control group, the T allele frequency was (74.2%) of rs12583006 in the BAFF gene while TT genotype frequency of rs12583006 in the BAFF gene was (57.8%). This is similar to the data acquired from multiple populations, such as the Gujarati Indians from Houston, Texas (74.3%), Sri Lankan Tamilians from the UK (77%), populations from Tuscany in Italy and the Iberian Peninsula in Spain (76.2%), populations with North and Western European ancestry (75.8%), a Bengali population from Bangladesh (73.8%), and a Punjabi population from Lahore, Pakistan (71.9%).[19]

The BAFF gene is a key regulator of B cell maturation and survival and is linked to several autoimmune disorders.[47-49] The rs12583006 variant can be found in the non-coding region of BAFF and has been associated with increased BAFF levels in patients with non-Hodgkin's lymphoma.[50,51]

Rs12583006 in the BAFF gene had a higher A allele frequency in the patient group than in the control group. This result is consistent with the findings of Nezos et al,[48] who reported that the mutant allele in the rs12583006 SNP of the BAFF gene was significantly associated with Caucasian Greek patients with pSS (low risk). A allele frequency in the patient was 35.1%, whereas A allele frequency in the control groups was 21.2%. The same study also compared the AA genotype between patients at low and high risk of developing pSS (such as those with B-cell lymphoma and peripheral neuropathy). The AA genotype frequency was lower in the pSS high-risk group than in the pSS low-risk group (18%), indicating the potential protective role of this allele against lymphoma development.[48] Theodorou et al[49] reported that the AA genotype increases susceptibility to lupus in patients with Athen's lupus and lupus-related plaque formation.

The current study has several limitations. For instance, the small number of patients with pSS does not accurately reflect the distribution of pSS within the general population. There is also some selection bias present, while the cross-sectional nature of the study is a further limitation. Despite its serological and clinical importance, we did not include cryoglobulin levels because they were not measured in most patients at the time of diagnosis. Future extensive studies are recommended to further support the conclusions of this study.

In conclusion, we demonstrated that the CT genotype of IL-1β at the –31 position (rs1143627) was protective against pSS development, whereas rs10488631 in IRF5 and rs12583006 in BAFF were associated with increased disease risk. We suggest that the cytokine genes of Saudi patients with pSS have a distinct genetic profile that plays a crucial role in pSS pathogenesis. These findings need to be validated in larger multicenter studies and further explored in different disease areas and populations.

Acknowledgment. *We thank the Deanship of Scientific Research at King Saud University for funding and supporting this research through the initiative of the DSR Graduate Students' Research Support. We thank all subjects for their cooperation and participation in this study. We also thank all the researchers, technicians, and nurses involved for their contributions. Lastly, we would like to thank Cambridge Proofreading LLC for the English language editing.*

References

1. Gøransson LG, Haldorsen K, Brun JG, Harboe E, Jonsson MV, Skarstein K, et al. The point prevalence of clinically relevant primary Sjögren's syndrome in 2 Norwegian counties. *Scand J Rheumatol* 2011; 40: 221-224.
2. Pers JO, Lahiri A, Tobón GJ, Youinou, P. Pathophysiological cytokine network in primary Sjögren's syndrome. *Presse Med* 2012; 41: e467-e474.
3. Björk A, Mofors J, Wahren-Herlenius, M. Environmental factors in the pathogenesis of primary Sjögren's syndrome. *J Intern Med* 2020; 287: 475-492.
4. Fletes-Rayas AL, Palafox-Sánchez CA, Muñoz-Valle JF, Orozco-Barocio G, Navarro-Hernández RE, Oregon-Romero, E.TNFR1-383 A>C polymorphism association with clinical manifestations in primary Sjögren's syndrome patients. *Genet Mol Res* 2016; 15.
5. Liu M, Wu X, Liu X, He J, Su Y, Guo J, Li Z. Contribution of dendritic cell immunoreceptor (DCIR) polymorphisms in susceptibility of systemic lupus erythematosus and primary Sjogren's syndrome. *Hum Immunol* 2015; 76: 808-811.
6. Magnusson V, Nakken B, Bolstad AI, Alarcón-Riquelme, ME. Cytokine polymorphisms in systemic lupus erythematosus and Sjogren's syndrome. *Scand J Immunol* 2001; 54: 55-61.
7. Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nat Genet* 2013; 45: 1284-1292.
8. Fogel O, Rivière E, Seror R, Nocturne G, Boudaoud S, Ly B, et al. Role of the IL-12/IL-35 balance in patients with Sjögren syndrome. *J Allergy Clin Immunol* 2018; 142: 258-268.
9. Imgenberg-Kreuz J, Rasmussen A, Sivils K, Nordmark G. Genetics and epigenetics in primary Sjögren's syndrome. *Rheumatology (Oxford)* 2021; 60: 2085-2098.

10. AlEnzi F, Alqahtani B, Alhamad EH, Daghestani M, Tashkandy Y, Othman N, et al. Fatigue in Saudi patients with primary Sjögren's syndrome and its correlation with disease characteristics and outcome measures: A cross-sectional study. *Open Access Rheumatol* 2020; 12: 303-308.
11. Omair MA, AlQahtani BS, AlHamad EH, Tashkandy YA, Othman NS, AlShahrani KA, et al. Disease phenotype and diagnostic delay in Saudi patients with primary Sjögren's syndrome. *Saudi Med J* 2021; 42: 405-410.
12. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2017; 76: 9-16.
13. Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T, et al. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). *Ann Rheum Dis* 2015; 74: 859-866.
14. Case control studies: Tests for deviation from Hardy-Weinberg equilibrium and tests for association. (n.d.). Institut für Humangenetik. [Accessed 2020 April 15]. Available from: https://ihg.gsf.de/cgi-bin/hw/hwa1.pl---
15. Nezos A, Mavragani CP. Contribution of genetic factors to Sjögren's syndrome and Sjögren's syndrome related lymphomagenesis. *J Immunol Res* 2015; 2015: 754825.
16. Papageorgiou A, Mavragani CP, Nezos A, Zintzaras E, Quartuccio L, De Vita S, et al. A BAFF receptor His159Tyr mutation in Sjögren's syndrome–related lymphoproliferation. *Arthritis Rheumatol* 2015; 67: 2732-2741.
17. Reksten TR, Lessard CJ, Sivils KL. Genetics in Sjögren syndrome. *Rheum Dis Clin North Am* 2016; 42: 435-447.
18. 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* 2015; 526: 68.
19. Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW. Interleukin-1β promotes oligodendrocyte death through glutamate excitetoxicity. *Ann Neurol* 2003; 53; 588-595.
20. Pociot F, Mølvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1β (IL-1β) gene correlates with IL-1β secretion in vitro. *Eur J Clin Investig* 1992; 22: 396-402.
21. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; 404: 398-402.
22. Ponomarenko MP, Suslov VV, Gunbin KV, Ponomarenko PM, Vishnevsky OV, Kolchanov NA. Identification of the relationship between the variability of the expression of signaling pathway genes in the human brain and the affinity of TATA-binding protein to their promoters. *Russ J Genet Appl Res* 2015; 5: 626-634.
23. Muraki Y, Tsutsumi A, Takahashi R, Suzuki E, Hayashi T, Chino Y, et al. Polymorphisms of IL-1 beta gene in Japanese patients with Sjögren's syndrome and systemic lupus erythematosus. *J Rheumatol* 2004; 31: 720-725.
24. Priya EK, Srinivas L, Rajesh S, Sasikala K, Banerjee M. Pro-inflammatory cytokine response pre-dominates immuno-genetic pathway in development of rheumatoid arthritis. *Mol Biol Rep* 2020; 47: 8669-8677.
25. Younes HEM, Ghonaim MM, Elkhyat AH, Labeeb AA, El-Hefnawy SM, Dawoud AM. Polymorphisms of interleukin-1β and cytotoxic T lymphocyte associated antigen-4 genes in systemic lupus erythematosus. *Egypt J Med Microbiol* 2019; 28.
26. Yin Y, Liu Y, Pan X, Chen R, Li P, Wu HJ, et al. Interleukin-1β promoter polymorphism enhances the risk of sleep disturbance in Alzheimer's disease. *PLoS One* 2016; 11: e0149945.
27. Yadav DK, Tripathi AK, Gupta D, Shukla S, Singh AK, Kumar A, et al. Interleukin-1B (IL-1B-31 and IL-1B-511) and interleukin-1 receptor antagonist (IL-1Ra) gene polymorphisms in primary immune thrombocytopenia. *Blood Res* 2017; 52: 264-269.
28. Mattuzzi S, Barbi S, Carletto A, Ravagnani V, Moore PS, Bambara LM, et al. (2007). Association of polymorphisms in the IL1B and IL2 genes with susceptibility and severity of systemic sclerosis. *J Rheumatol* 2007; 34: 997-1004.
29. Mohammadoo-Khorasani M, Salimi S, Tabatabai E, Sandoughi M, Zakeri Z, Farajian-Mashhadi F. Interleukin-1β (IL-1β) & IL-4 gene polymorphisms in patients with systemic lupus erythematosus (SLE) & their association with susceptibility to SLE. *Indian J Med Res* 2016; 143: 591.
30. Fu LY, Qiu X, Deng QL, Huang P, Pi L, Xu Y, et al. The IL-1B gene polymorphisms rs16944 and rs1143627 contribute to an increased risk of coronary artery lesions in Southern Chinese Children with Kawasaki disease. *J Immunol Res* 2019; 2019: 4730507.
31. Lee KA, Ki CS, Kim HJ, Sohn KM, Kim JW, Kang WK, et al. Novel interleukin 1β polymorphism increased the risk of gastric cancer in a Korean population. *J Gastroenterol* 2004; 39: 429-433.
32. Wang L, Zhao W, Hong J, Niu F, Li J, Zhang S, et al. Association between IL1B gene and cervical cancer susceptibility in Chinese Uygur Population: A case-control study. *Mol Genet Genomic Med* 2019; 7: e779.
33. Tak KH, Yu GI, Lee MY, Shin DH. Association between polymorphisms of interleukin 1 family genes and hepatocellular carcinoma. *Med Sci Monit* 2018; 24: 3488.
34. Yencilek F, Yildirim A, Yilmaz SG, Altinkilic EM, Dalan AB, Bastug Y, et al. Investigation of interleukin-1β polymorphisms in prostate cancer. *Anticancer Res* 2015; 35: 6057-6061.
35. Liu X, Wang Z, Yu J, Lei G, Wang S, et al. Three polymorphisms in interleukin-1β gene and risk for breast cancer: a meta-analysis. *Breast Cancer Res Treat* 2010; 124: 821-825.
36. Nordmark G, Kristjansdottir G, Theander E, Eriksson P, Brun JG, Wang C, et al. Additive effects of the major risk alleles of IRF5 and STAT4 in primary Sjögren's syndrome. *Genes Immun* 2009; 10: 68-76.
37. Imgenberg-Kreuz J, Sandling JK, Almlöf JC, Nordlund J, Signér L, Norheim KB, et al. Genome-wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. *Ann Rheum Dis* 2016; 75: 2029-2036.
38. Sigurdsson S, Göring HH, Kristjansdottir G, Milani L, Nordmark G, Sandling JK, et al. (2008). Comprehensive evaluation of the genetic variants of interferon regulatory factor 5 (IRF5) reveals a novel 5 bp length polymorphism as strong risk factor for systemic lupus erythematosus. *Human Molecular Genetics* 2008; 17: 872-881.
39. Ferreiro-Neira I, Calaza M, Alonso-Perez E, Marchini M, Scorza R, Sebastiani GD, et al (2007). Opposed independent effects and epistasis in the complex association of IRF 5 to SLE. *Genes & Immunity* 2007; 8:429-438.
40. Taylor KE, Chung SA, Graham RR, Ortmann WA, Lee AT, Langefeld CD, et al. Risk alleles for systemic lupus erythematosus in a large case-control collection and associations with clinical subphenotypes. *PLoS Genetics* 2011; 7: e1001311.

41. Alarcón-Riquelme ME, Ziegler JT, Molineros J, Howard TD, Moreno-Estrada A, Sánchez-Rodríguez E, et al. Genome-wide association study in an Amerindian ancestry population reveals novel systemic lupus erythematosus risk loci and the role of European admixture. ***Arthritis Rheumatol*** 2016; 68: 932-943.
42. Zervou MI, Dorschner JM, Ghodke-Puranik Y, Boumpas DT, Niewold TB, Goulielmos GN. Association of IRF5 polymorphisms with increased risk for systemic lupus erythematosus in population of Crete, a southern-eastern European Greek island. ***Gene*** 2017; 610: 9-14.
43. Carmona FD, Onat AM, Fernández-Aranguren T, Serrano-Fernández A, Robledo G, Direskeneli H, et al. J Analysis of systemic sclerosis-associated genes in a Turkish population. ***J Rheumatol*** 2016; 43: 1376-1379.
44. Graham RR, Hom G, Ortmann W, Behrens TW. Review of recent genome-wide association scans in lupus. ***J Intern Med*** 2009; 265: 680-688.
45. Elghzaly AA, Metwally SS, El-Chennawi FA, Elgayaar MA, Mosaad YM, El-Toraby EE, et al. IRF5, PTPN22, CD28, IL2RA, KIF5A, BLK and TNFAIP3 genes polymorphisms and lupus susceptibility in a cohort from the Egypt Delta; relation to other ethnic groups. ***Hum Immunol*** 2015; 76: 525-531.
46. Stock CJ, De Lauretis A, Visca D, Daccord C, Kokosi M, Kouranos V, et al. Defining genetic risk factors for scleroderma-associated interstitial lung disease. ***Clin Rheumatol*** 2020; 39: 1173–1179.
47. Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, et al. BAFF mediates survival of peripheral immature B lymphocytes. ***J Exp Med*** 2000: 192: 1453-1466.
48. Nezos A, Papageorgiou A, Fragoulis G, Ioakeimidis D, Koutsilieris M, Tzioufas AG. B-cell activating factor genetic variants in lymphomagenesis associated with primary Sjogren's syndrome. ***J Autoimmun*** 2014; 51: 89-98.
49. Theodorou E, Nezos A, Antypa E, Ioakeimidis D, Koutsilieris M, Tektonidou M, et al. B-cell activating factor and related genetic variants in lupus related atherosclerosis. ***J Autoimmun*** 2018; 92: 87-92.
50. Novak AJ, Slager SL, Fredericksen ZS, Wang AH, Manske MM, Ziesmer S, et al. Genetic variation in B-cell–activating factor is associated with an increased risk of developing B-cell non–Hodgkin lymphoma. ***Cancer Res*** 2009; 69: 4217-4224.
51. Han Q, Yang C, Li N, Li F, Sang J, Lv Y, et al. Association of genetic variation in B-cell activating factor with chronic hepatitis B virus infection. ***Immunol Lett*** 2017; 188: 53-58.