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ORIGINAL ARTICLE

Interactions between TNFAIP3, PTPN22, and TRAF1-C5 gene polymorphisms in patients with primary Sjögren's syndrome

Daniel Cadena-Sandoval[1], Isela Montúfar-Robles[2], Rosa Elda Barbosa-Cobos[5], Gabriela Hernández-Molina[6], Ana Karen Salas-García[5], Norma Sánchez-Zauco[3], Julian Ramírez-Bello[4]

[1]*Universidad Juárez Autónoma De Tabasco, Comalcalco Multidisciplinary Academic Division, Comalcalco, Tabasco, Mexico*
[2]*División De Investigación, Hospital Juárez De México, Ciudad De México, Mexico*
[3]*División De Diagnostico Y Tratamientos Auxiliares, Centro Médico Nacional Siglo Xxi, Ciudad De México, Mexico*
[4]*Subdirección de Investigación Clínica, Instituto Nacional De Cardiologia Ignacio Chávez, Ciudad De Mexico, Mexico*
[5]*Departamento De Reumatología, Hospital Juárez De México, Ciudad De México, Mexico*
[6]*Departamento De Inmunología Y Reumatología, Instituto Nacional De Ciencias Médicas Y Nutrición, Ciudad De México , Mexico*

Correspondence: Julian Ramírez-Bello, MD.
E-mail: dr.julian.ramirez.inc@gmail.com

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ABSTRACT

Objectives: The aim of our study was to investigate whether TNFAIP3, PTPN22, and TRAF1-5 single nucleotide polymorphisms (SNPs) are associated with susceptibility, severity, or serological markers in primary Sjögren's syndrome (pSS).

Patients and methods: The cases and controls study was conducted between December 2021 and June 2022. TNFAIP3 rs10499194C/T, rs6920220G/A, and rs2230926T/G, PTPN22 rs2476601C/T and rs33996649G/A, and TRAF1-C5 rs10818488G/A polymorphisms were genotyped in 154 female pSS patients (mean age: 45.2±6.8 years) and 313 female control subjects (mean age: 50.3±7.5 years) using the TaqMan® SNP genotyping assay. An association analysis between TNFAIP3, PTPN22, and TRAF1-C5 SNPs and susceptibility, clinical characteristics, and serological markers of pSS was performed. Interactions between TNFAIP3, PTPN22, and TRAF1-C5 SNPs were also evaluated in patients and controls.

Results: The genotype and allele frequencies showed no association with susceptibility, severity, or serological markers of pSS. Nevertheless, several interactions between TNFAIP3 and TRAF1-C5 or TNFAIP3, PTPN22, and TRAF1-C5 genotypes were associated with susceptibility to pSS ($p<0.01$).

Conclusion: Individual TNFAIP3, PTPN22, and TRAF1-C5 SNPs are not associated with susceptibility, severity, or serological markers of pSS. However, genetic interactions between TRAF1-C5 and TNFAIP3 or TNFAIP3, PTPN22, and TRAF1-C5 SNPs are risk factors for pSS.

Keywords: Genetic interaction, primary Sjögren's syndrome, PTPN22, TNFAIP3, TRAF1-C5.

Sjögren's syndrome (SS) is a chronic systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands, dry eye, dry mouth, and extraglandular systemic findings.[1] SS may present as an entity by itself, called primary SS (pSS), or may be secondary to other autoimmune diseases (ADs), such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or systemic sclerosis.[1,2] The etiology of pSS is not entirely known, but similar to other ADs, it results from the combined effects of genetic and environmental risk factors. Genetic studies on pSS relied heavily on extrapolations from SLE and RA until the advent of genome-wide association studies (GWASs) in this AD of exocrine glands,[3] which have been conducted primarily in Caucasian and Asian populations; however, due to the low number of GWASs conducted on patients with pSS, a few genes, such as HLA-II, TNFAIP3 (tumor necrosis factor-alpha-induced protein 3), BLK (B-lymphoid tyrosine kinase), STAT4 (signal transducer and activator of transcription 4), GTF2I (general transcription factor IIi), RBMS3 (ribonucleic acid-binding motif single-stranded interacting protein 3), and IRF5 (interferon regulatory factor 5), among others, have been identified to be associated with this AD.[4-7] Therefore, a limited number of single nucleotide

polymorphisms (SNPs) located in these genes have been associated with this AD.[8-12]

Genome-wide association studies have identified several susceptibility loci implicated in a broad array of biological pathways, and the association of certain HLA and non-HLA loci with different ADs has been consistently reported.[4-7,13] For example, PTPN22 (protein tyrosine phosphatase nonreceptor type 22) is an important non-HLA genetic risk factor for RA, SLE, and Graves' disease.[14,15] Thus, PTPN22, BLK, BANK1 (B-cell scaffold protein with ankyrin repeats 1), TNFAIP3, and TRAF1-C5 (tumor necrosis factor receptor associated factor 1-complement component), among others, have been identified as shared risk factors for different ADs.[12-20] However, because these findings have not been replicated in all populations, we aimed to determine the possible roles of the TNFAIP3 rs10499194C/T, rs6920220G/A, and rs2230926T/G; PTPN22 rs2476601C/T and rs33996649G/A; and TRAF1-C5 rs10818488G/A polymorphisms in pSS susceptibility. In addition, we also evaluated interactions between PTPN22, TNFAIP3, and TRAF1-C5 genotypes in a Mexican population with pSS.

PATIENTS AND METHODS

The cases and controls study was performed on 154 female pSS patients (mean age: 45.2±6.8 years) from the Service of Rheumatology of the Hospital Juárez de México and from the Department of Immunology and Rheumatology of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán between December 2021 and June 2022. Three hundred thirteen healthy females (mean age: 50.3±7.5 years) were included as a control group. Patients were diagnosed with pSS according to the 2002 American European Consensus Group classification criteria for pSS.[21] Because the presence of other ADs, such as SLE or RA, could affect the allele and genotypic frequencies of SNPs evaluated in our study, we decided to include individuals who exclusively presented pSS. Clinical, laboratory, and treatment data were obtained from the patients' medical records. All our patients and control subjects were born in Mexico, and they were from Mexico City, the State of Mexico, Hidalgo, and Querétaro. Both patients and controls were unrelated Mexican-Mestizo women aged greater than 18 years old. All controls had no family history of ADs or inflammatory diseases, such as asthma, obesity, or type 2 diabetes.

Deoxyribonucleic acid extraction

Blood samples were obtained from patients and control subjects, which were immediately stored at −4°C and deoxyribonucleic acid (DNA) was isolated within 48 h; otherwise, once blood samples were obtained, DNA was isolated immediately using the standard phenol-chloroform technique, then DNA concentration and purity was assessed with a SimpliNano™ Spectrophotometer (Biochrom, Cambridge, UK). DNA samples with an absorbance ratio of 260/280 nm ranging from 1.8 to 2.0 and concentrations of ≥50 ng/μL were considered appropriate for the study.

Genotyping

An allelic discrimination assay was performed with the following TaqMan probes: TNFAIP3 rs10499194C/T (C___1575581_10), rs6920220G/A (C___1575581_10), and rs2230926T/G (C___7701116_10), PTPN22 rs2476601C/T (C__16021387_20) and rs33996649G/A (C__25937239_30), and TRAF1-C5 rs10818488G/A (C___2783655_10). The CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) was used to obtain fluorescence emission and the allelic discrimination plot. Polymerase chain reaction (PCR) conditions for each amplicon of PTPN22, TNFAIP3, and TRAF1-C5 were as follows: 10 ng of DNA per sample, 2.5 μL of TaqMan® Universal Master Mix (2×) (Applied Biosystems, Foster City, CA, USA), 2.435 μL of nuclease-free water, and 0.065 μL of TaqMan probes (Applied Biosystems, Foster City, CA, USA). The PCR conditions used to obtain the amplicons were as follows: pre-PCR (one cycle) at 50°C for 2 min and at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 sec, and annealing and extension at 60°C for 1 min.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was applied to evaluate the genotype distribution

of the six PTPN22, TNFAIP3, and TRAF1-C5 polymorphisms in controls. Finetti software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to evaluate both the HWE and the genetic association between all variants and susceptibility to pSS. The search for the association of clinical symptoms and serological markers with the disease was performed using GraphPad Prism version 9.1.2 software (GraphPad Software, San Diego, California USA). Linkage disequilibrium (LD) between TNFAIP3 and PTPN22 SNPs, as well as haplotype frequencies, were estimated using Haploview 4.2 software (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). Multifactor dimensionality reduction software v3.0.2 (http://sourceforge.net/projects/mdr/) was used to analyze interactions between the SNPs evaluated. This is a model-free method, that is, the multifactor dimensionality reduction does not assume any genetic model. A p-value <0.05 was considered statistically significant.

RESULTS

Of the 154 pSS patients, 87% and 52.8% were positive for anti-SSA (Ro) and anti-SSB (La) antibodies, respectively. The most common extraglandular manifestation in our group of pSS patients was arthritis, followed by Raynaud's phenomenon, and the least common presentations were myositis and interstitial lung disease. Almost all patients required ocular symptomatic treatment, and more than 50% required oral symptomatic and systemic treatment (Table 1).

Hardy-Weinberg equilibrium and analysis of the association of PTPN22, TNFAIP3, and TRAF1-C5 SNPs between patients and controls

The genotype distribution was in HWE in both patients and controls (p>0.05). The genotype and allele frequencies of the six TNFAIP3, PTPN22, and TRAF1-C5 variants were compared between patients and controls. As the frequencies of the six variants were similar between patients and controls, we found no association with susceptibility (Table 2). In addition, we also identified similar allelic and genotypic frequencies of the six PTPN22, TNFAIP3, and TRAF1-C5 SNPs among patients who presented myositis, arthritis, vasculitis, Raynaud's phenomenon, weight loss, salivary flow, and anti-SSA and anti-SSB antibodies against those who did not present these glandular and extraglandular manifestation or anti-SSA and anti-SSB antibodies (data not shown). Thus, our data suggested that these PTPN22, TNFAIP3, and TRAF1-C5 polymorphisms were not associated with the severity of pSS or with the presence of anti-SSA or anti-SSB antibodies.

Haplotypes and LD of PTPN22 and TNFAIP3 in patients and controls

The allelic combination of the TNFAIP3 and PTPN22 SNPs showed three haplotypes in each

Table 1. Clinical symptoms, serological markers, and medical treatment modalities of patients with pSS

Variable	Frequency	%
Schirmer's test*	133	82.6
Rose Bengal score§	83	51.6
Unstimulated whole saliva flow rate‡	140	87
Anti-SSA (Ro)	140	87
Anti-SSB (La)	85	52.8
Labial salivary gland biopsy&		
Positive	121	75.2
Negative	5	3.1
Not performed	35	21.7
Extra-glandular manifestations		
Fever	14	8.7
Weight loss	13	8.1
Arthritis	43	26.7
Myositis	2	1.2
Vasculitis	11	6.83
Raynaud phenomenon	18	11.2
Interstitial lung disease	8	5
Symptomatic treatment		
Ocular	147	91.3
Oral	76	47.2
Systemic treatment		
Hydroxicholoroquine	83	51.6
Methotrexate	51	31.7
Cyclophosphamide	4	2.5
Rituximab	2	1.2
Mycophenolate	3	1.9
Cyclosporine	1	0.62
Glucocorticoids	67	41.6
Comorbidity		
Diabetes	12	7.5
Hypertension	38	23.6
Dyslipidemia	39	24.4
Smoking	39	24.2

pSS: Primary Sjögren's syndrome; * <5 mm in 5 min; § ≥4 according to van Bijsterveld's scoring system; ‡ ≤0.1 ml/min; & With focal lymphocytic sialadenitis and focus score ≥1.

Table 2. Genotypic and allelic frequencies of the TNFAIP3, PTPN22, and TRAF1-C5 SNPs, and association analysis in patients with pSS and control subjects

SNPs	Model	Genotype or allele	pSS		Controls		OR	95% CI	*p*
			n	%	n	%			
TNFAIP3 rs10499194C/T	Codominant	CC	70	45.5	125	40.1			
		CT	64	41.5	147	46.8	0.8	0.51-1.18	NS
		TT	20	13.0	41	13.1	0.8	0.48-1.61	NS
	Allele	C	204	66.2	399	63.5			
		T	104	33.8	229	36.5	0.8	0.67-1.16	NS
rs6920220G/A	Codominant	GG	135	87.7	278	88.8			
		GA	19	12.3	33	10.5	1.2	0.65-2.15	NS
		AA	0	0.0	2	0.7	0.4	0.02-8.62	NS
	Allele	G	289	93.8	589	94.1			
		A	19	6.2	37	5.9	1.0	0.59-1.85	NS
rs2230926T/G	Codominant	TT	151	98.1	304	97.1			
		TG	3	1.9	9	2.9	0.7	0.18-2.51	NS
		GG	0	0.0	0	0.0	2.0	0.04-101.78	NS
	Allele	T	305	99.0	617	98.6			
		G	3	1.0	9	1.4	0.7	0.18 - 2.51	NS
PTPN22 rs2476601C/T	Codominant	CC	149	96.8	307	98.1			
		CT	5	3.2	6	1.9	1.7	0.52-5.72	NS
		TT	0	0.0	0	0.0	2.0	2.04-104.16	NS
	Allele	C	303	98.4	620	99.0			
		T	5	1.6	6	1.0	1.7	0.52-5.63	NS
rs33996649G/A	Codominant	GG	152	98.7	302	96.5			
		GA	2	1.3	11	3.5	0.4	0.08-1.65	NS
		AA	0	0.0	0	0.0	2.0	0.04-100.45	NS
	Allele	G	306	99.4	615	99.0			
		A	2	0.6	11	1.0	0.4	0.08-1.66	NS
TRAF1-C5 rs10818488G/A	Codominant	GG	57	37.0	127	40.6			
		GA	78	50.7	136	43.5	1.3	0.84-1.94	NS
		AA	19	12.3	50	15.9	0.8	0.46-1.56	NS
	Allele	G	192	62.3	390	62.3			
		A	116	37.7	236	37.7	1.0	0.75-1.32	NS

SNPs: Single nucleotide polymorphisms; pSS: Primary Sjögren syndrome; OR: Odds ratio; CI: Confidence interval; NS: Not significant.

gene with similar frequencies between both patients and controls; thus, no haplotype showed an association with susceptibility to pSS (data not shown). None of the PTPN22 and TNFAIP3 variants were in LD (data not shown).

Genetic interactions between PTPN22, TNFAIP3, and TRAF1-C5 genotypes and pSS

Since we did not identify an individual association of PTPN22, TNFAIP3, and

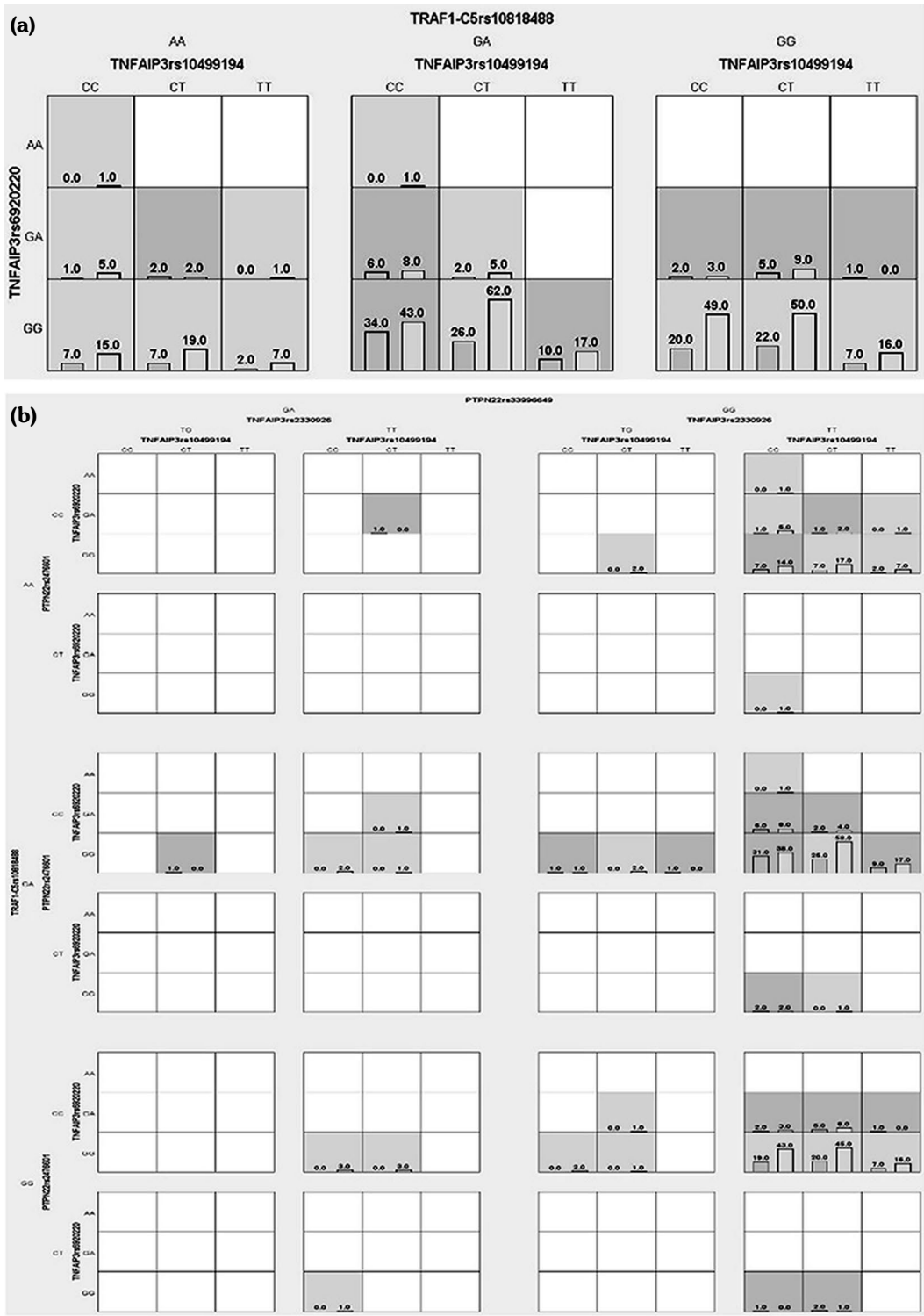

Figure 1. Illustrates the distribution of genotypes for PTPN22, TNFAIP3, and TRAF1-C5 in cases and controls. **(a)** Displays the distribution of TNFAIP3 and TRAF1-C5 genotypes among cases and controls. **(b)** The distribution of genotypes for PTPN22, TNFAIP3, and TRAF1-C5 is depicted in both cases and controls. The figure highlights a pattern of high-risk (dark gray) and low-risk (light gray) genotype combinations observed in cases and controls. Due to the nonlinear nature of genotypic combinations across each multilocus dimension, potential epistasis or gene-gene interactions can be discerned.

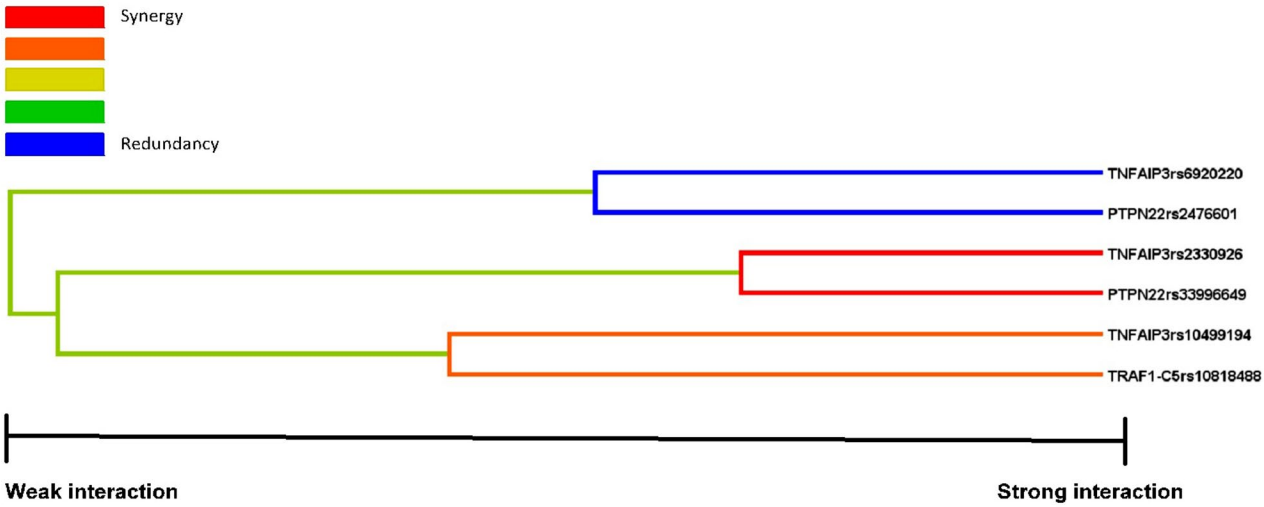

Figure 2. Presents a dendrogram estimating interaction information or entropy for each SNP included in our study. Our data reveal redundant, independent, and synergistic interactions. Specifically, a synergistic interaction is observed between two non-synonymous SNPs, namely PTPN22 (R263Q) and TNFAIP3 (P127C). Notably, PTPN22 and TNFAIP3 represent two loci strongly associated with different autoimmune diseases.

TRAF1-C5 SNPs or by PTPN22 and TNFAIP3 haplotypes, we decided to perform a gene-gene interaction analysis and identified genotype distribution between these three loci in cases and controls (Figure 1). After analysis, we identified redundant, independent, and synergistic interactions (Figure 2). Of note, a synergistic interaction was observed between the nonsynonymous SNPs of PTPN22 and TNFAIP3. Unexpectedly, we identified several interactions associated with susceptibility for pSS (Table 3). Considering the highest testing accuracy and the cross-validation consistency, our best model of interaction was TNFAIP3 rs10499194-rs6920220, PTPN22 rs2476601-rs33996649, and TRAF1-C5 rs10818488 (testing accuracy 0.5064, cross-validation 10/10, odds ratio=1.9, and an interaction p-value=0.001). In addition, we also identified other interactions between TNFAIP3 and

Table 3. Gene-gene interaction models between TNFAIP3, PTPN22, and TRAF1-C5 SNPs in patients with pSS and control subjects

Number of factors	Models	Training accuracy	Testing accuracy	CVC	*p*	OR	95% CI
1	TRAF1-C5 rs10818488	0.5371	0.5086	8/10	NS	1.3	0.91-1.97
2	TNFAIP3 rs10499194-TRAF1-C5 rs10818488	0.555	0.5091	9/10	0.02	1.6	1.07-2.44
3	TNFAIP3 rs10499194-rs6920220-TRAF1-C5 rs10818488	0.5663	0.5093	9/10	0.005	1.8	1.19-2.71
4	TNFAIP3 rs10499194-rs6920220-PTPN22 rs2476601-TRAF1-C5 rs10818488	0.5739	0.4919	7/10	0.003	1.8	1.23-2.72
5	TNFAIP3 rs10499194-rs6920220-PTPN22 rs2476601-rs33996649-TRAF1-C5 rs10818488	0.5802	0.5064	10/10	0.001	1.9	1.23-2.85
6	TNFAIP3 rs10499194-rs6920220-rs2330926-PTPN22 rs2476601-rs33996649-TRAF1-C5 rs10818488	0.5836	0.5016	10/10	0.0007	2.0	1.33-2.94

SNPs: Single nucleotide polymorphisms; pSS: Primary Sjögren's syndrome; CVC: Cross-validation consistency; OR: Odds ratio; CI: Confidence interval; NS: Not significant;

TRAF1-C5 or TNFAIP3, PTPN22, and TRAF1-C5 genotypes with susceptibility for pSS (Table 3).

DISCUSSION

Autoimmune diseases could share clinical similarities, presence of autoantibodies, a higher prevalence in females,[22] and susceptibility genes, such as BLK, BANK1, PTPN22, and TNFAIP3, among others.[12-20] From the point of view of genetic susceptibility, we have recently reported associations of PTPN22 SNPs with RA, SLE, and Graves' disease in patients from Mexico.[14,15] PTPN22 is a risk factor for RA, SLE, and pSS, primarily in Caucasian and Latin American populations;[14,15,23-26] meanwhile, TNFAIP3 is a risk factor for RA, SLE, and pSS, mainly in Asian and Caucasian populations.[4,7,18,27,28] Finally, TRAF1-C5 has been reported to be a risk factor for RA and SLE in Africans and Caucasians.[20,29,30] Because ADs shared some susceptibility loci, we decided to evaluate six variants in these three genes scarcely studied in pSS[4,7,18,31,32] in a sample of Mexican patients with this AD.

TNFAIP3 encodes the A20 protein, a negative regulator of TNF (tumor necrosis factor)-induced NF-κB (nuclear factor kappa B) signaling.[27] Different TNFAIP3 SNPs have previously been associated with the susceptibility to pSS in Han Chinese or in European/European-derived populations.[4,11,18,28] However, other studies conducted in these same populations have not replicated this association.[5,6,9,33-35] The most frequently evaluated variant of TNFAIP3, the rs2230926T/G (Phe127Cys) SNP, has been associated with pSS in Greek and Caucasian patients from the USA,[11,28] but not in Italian,[18] Israeli,[31] Chinese,[33] French/British,[34] and Swedish/Norwegian patients.[35] Only one study performed in Italy reported an association between the TNFAIP3 rs6920220G/A variant and pSS.[18] Our results were consistent with those findings of no association between the TNFAIP3 rs2230926T/G and rs6920220G/A variants and susceptibility to pSS. The discrepancy in the results might be attributed to the sample size and the ancestry of each population as previous studies have been conducted in Asian and Caucasian patients, while the current study was conducted in a sample of controls and pSS patients from Mexico, composed of a mixture of Amerindians, Spaniards, and Africans.[36] Notably, rs2230926T/G has been reported to be related to pSS-associated lymphoma but not to pSS in French, British, and Italian populations.[18,34,37] Thus, these studies suggest that TNFAIP3 rs2230926T/G and possibly other polymorphisms in the same gene might be associated with the severity of pSS. Other studies have reported that TNFAIP3 rs2230926T/G is not a severity factor for oral and ocular dryness, arthritis, and Raynaud's phenomenon and is not associated with the presence of anti-SSA and anti-SSB antibodies.[11,35] These results agree with the findings of our study. To the best of our knowledge, the TNFAIP3 rs10499194C/T variant has not been studied in patients with pSS, and no GWAS has reported its association in any population. Our data suggest that TNFAIP3 is not a risk factor for pSS, at least in Mexican patients, but more studies should be conducted in other Latin American populations to determine whether these or other TNFAIP3 polymorphisms are important in pSS.

Additionally, we evaluated three PTPN22 and TRAF1-C5 SNPs. The PTPN22 rs2476601C/T variant has been associated with pSS only in a Colombian population;[26] however, Ittah et al.[32] did not replicate this finding in a French population. Another study of 265 multiplex autoimmune families also reported no association.[38] Our results are consistent with these previous findings that showed no association between PTPN22 rs2476601C/T and pSS. Discrepancies between studies include sample size and ancestry. The PTPN22 rs33996649G/A and TRAF1-C5 rs10818488G/A variants, to our knowledge, have not been reported to be associated with pSS, and our data show that both variants are not associated with this AD. Moreover, we determined the role of these six PTPN22, TNFAIP3, and TRAF1-C5 SNPs in the severity of pSS; therefore, we evaluated whether the allelic and genotypic frequencies of these polymorphisms were different among patients who presented extraglandular traits, such as vasculitis, arthritis, and Raynaud's phenomenon, compared to those who did not present extraglandular damage. We also compared patients positive

for anti-SSA and anti-SSB antibodies compared to those who were negative. After analysis, no statistically significant differences and no associations were identified between PTPN22, TNFAIP3, and TRAF1-C5 polymorphisms and clinical symptoms or anti-SSA and anti-SSB antibodies. However, it is important to note that identifying genetically susceptible individuals could contribute to having preventive, predictive, and personalized medicine in pSS. Similar results were reported between PTPN22 R620W (rs2476601C/T) and clinical symptoms or with the presence of anti-SSA and anti-SSB antibodies in patients with pSS from Colombia; thus, it was not a risk factor associated with severity or serological markers in Colombians.[26] As far as we can tell, no study has reported the allelic and genotypic frequencies of TRAF1-C5 polymorphisms and their association with the severity of pSS or with the presence of anti-SSA and anti-SSB antibodies.

Finally, some studies have shown the importance of interactions between genotypes of SNPs with various ADs, as has been observed between BLK and BANK1 genotypes in patients with SLE, RA, and pSS.[12,16,17] We recently reported some interactions associated with susceptibility for pSS between BLK-associated genotypes and non-BANK1-associated genotypes.[12] Therefore, we performed an interaction analysis between TNFAIP3, PTPN22, and TRAF1-C5 genotypes, identifying that some of them are associated with the risk of pSS. The best model of interactions was TNFAIP3 rs10499194C/T-rs6920220, PTPN22 rs2476601-rs3399664-, and TRAF1-C5 rs10818488G/A, which indicates a combined effect of TNFAIP3, PTPN22, and TRAF1-C5 genotypes on susceptibility to pSS. These interactions are based on three genes that individually show no association with this disease. This information is important since SNPs that individually showed no association with a disease should be evaluated through analysis of gene interactions.[12,16,17] From a functional perspective, rs10499194G/A alters TNFAIP3 mRNA (messenger ribonucleic acid) levels in peripheral blood mononuclear cells.[39] On the other hand, the TNFAIP3 rs2230926G allele (127Cys) leads to lower TNF-induced NF-κB inhibitory activity compared to the T allele (Phe127). This decreased anti-inflammatory activity of A20 could increase NF-κB activity, leading to the release of proinflammatory cytokines in response to TNF.[27] Both SNPs of PTPN22 (encodes the lymphoid-specific tyrosine phosphatase [LYP] protein, a negative regulator of T cell activation), rs2476601C/T (R620W) and rs33996649G/A (R263Q), affect the LYP signaling pathway and T cell activation.[23,40] The TRAF1-C5 rs10818488G/A polymorphism has been reported to result in a significant difference in the TRAF1 transcription level between phorbol myristate acetate-stimulated human lymphoblastoid cell lines from risk allele carriers and noncarriers.[41] Thus, these interactions might affect the expression levels of TNFAIP3, PTPN22, and TRAF1-C5 or the activity of A20 and LYP, increasing the susceptibility for pSS. Although we have identified some interactions between TNFAIP3, PTPN22, and TRAF1-C5 genotypes and susceptibility to pSS, other studies should be performed in different populations to replicate our results. In addition, further studies should be carried out to determine whether the genotypes of TNFAIP3, PTPN22, and TRAF1-C5 interactions associated with pSS might affect the expression at the mRNA or protein level in patients with this AD.

There are some limitations to our study. Only six TNFAIP3, PTPN22, and TRAF1-C5 SNPs were selected, while other variants have been associated with other ADs, and some of them might be associated with pSS but were not analyzed here. There was an absence of informative markers of ancestry to determine the Amerindian, European, and African components in our group of patients and controls; therefore, our results should be taken with caution.

In conclusion, this study is the first to evaluate the association of TNFAIP3 and TRAF1-C5 SNPs with pSS in a Mexican population. The results suggest that TNFAIP3 rs10499194C/T, rs6920220G/A, and rs2230926T/G, PTPN22 rs2476601C/T and rs33996649G/A, as well as TRAF1-C5 rs10818488G/A are not individually associated with susceptibility, severity, or serological markers of pSS. However, interactions between TNFAIP3 and TRAF1-C5 or TNFAIP3, PTPN22, and TRAF1-C5 genotypes are associated with the susceptibility to pSS.

Ethics Committee Approval: The study protocol was approved by the Juárez de México Hospital Ethics Committee (date: September 2021, no: HJM 020/21-I). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Implemented the experiments, participated in data analysis, and wrote the draft of the manuscript: D.C.S.; Implemented the experiments and contributed to data analysis: I.M.R.; Participated in the recruitment of patients with pSS and contributed to data analysis: R.E.B.C., G.H.M.; Was responsible for the conception and design of the experiments, participated in data analysis, and contributed to the review and final writing of the manuscript: J.R.B.; Implemented the experiments and contributed to data analysis: N.S.Z.; Participated in the recruitment of patients with pSS and contributed to data analysis: A.K.S.G.

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