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RESEARCH ARTICLE

PTPN22 rs2488457C>G and *TRAF1-C5* rs10818488A>G and rs3761847G>A variants in Mexican mestizo women with Systemic Lupus Erythematosus

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ABSTRACT

Introduction: Systemic lupus erythematosus is an autoimmune disease with higher prevalence in women. Single nucleotide variants in genes involved in the regulation of autoreactive cells, such as *PTPN22* rs2488457C>G, and *TRAF1-C5* rs10818488A>G and rs3761847G>A, have been associated with the disease in some populations; however, little is known about these variants in the Mexican mestizo population.

Aim: We analyzed whether these variants are associated with lupus in women from Central Mexico and Yucatan.

Methods: DNA samples from two hundred female patients with lupus (100 from Yucatan and 100 from Central Mexico) and 200 female healthy controls (100 from Yucatan and 100 from Central Mexico) were genotyped. Allelic and genotypic frequencies of variants were calculated and their association with lupus was analyzed.

Results: Distribution of risk allele *PTPN22* rs2488457G ranged 36% to 48%, while *TRAF1-C5* rs10818488A and rs3761847G ranged from 34% to 40%. Heterozygous C/G was the most frequent for *PTPN22* rs2488457 in all studied groups, while *TRAF1-C5* heterozygous genotype was the most frequent in cases of Yucatan and controls from Central Mexico. However, we did not find significant differences in allelic and genotypic frequencies of *PTPN22* and *TRAF1-C5* variants, neither its haplotypes between cases and controls, suggesting a lack of association for lupus in the two Mexican populations.

Conclusion: The *PTPN22* rs2488457C>G and *TRAF1-C5* rs10818488A>G and rs3761847G>A variants do not confer susceptibility with the development of lupus in both studied populations. However, the strong linkage disequilibrium observed in the *TRAF1-C5* haplotypes suggests that they are co-inherited together and could be involved in the development of the disease in association with other genes or risk factors, as well as the Caucasian influence.

Keywords: Systemic lupus erythematosus, *PTPN22, TRAF1-C5*, Variants, Yucatan, Mexico Central.

Introduction.

Systemic lupus erythematosus (SLE) is an inflammatory systemic autoimmune disease (AD) of multifactorial etiology. [1-4] It predominantly affects female gender, with variable incidence and prevalence depending on the population and geographic area.[5-8] In Mexico, its incidence is unknown, but a national prevalence of 0.06% (0.08% women vs 0.04% men) has been reported; in Yucatan it is about 0.07% and in Mexico City 0.09%.[9, 10]

Genome-wide association studies (GWASs) allowed the identification of many loci associated with SLE susceptibility.[11, 12] *PTPN22* (1p13.2) gene encodes the lymphoid tyrosine phosphatase (Lyp) protein involved in T/B cell signaling as an inhibitor of T cell and B cell receptor signaling. This protein causes dephosphorylation of Lck (Tyr-394) and ZAP70 (Tyr-493) that inhibits T cell activation. Lyp also mediates dephosphorylation of Syk and PCLγ2 and inhibits B cell activation.[13-18] Studies have found that some *PTPN22* single nucleotide variations (SNVs) are associated with SLE, for example, the rs2476601C>T variant in exon 14, result in the substitution of arginine to tryptophan at codon 620 (R620W), and causing the interruption of T cell receptor signaling. Decreased expression of Lyp increases phosphorylation of signaling proteins leading to the continued activation of autoreactive T and B cells, contributing to the pathogenesis of ADs such as SLE. Weaker T-cell signaling due to increased Lyp activity could result in failure to delete autoreactive T-cells during thymic selection. Alternatively, increased Lyp function may inhibit regulatory T-cell activity, resulting in immune responses against autoantigens.[19] The rs2488457C>G variant located in the 50-promoter region of *PTPN22*, it has associated with rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), type 1 diabetes (T1D), latent autoimmune diabetes, and SLE, although its function has not yet been characterized.[20-25]

TRAF1/C5 (9q33-34) gene has been associated with RA, JIA, and SLE.[26-29] TRAF1 is a member of the TRAF protein family, involved in signaling through binding to receptors of the TNF superfamily. It has also been involved in negative regulation of the T cells activation and proliferation, inhibiting the production of proinflammatory cytokines such as TNF-α.[30-33] *C5* is a central component of the classical complement pathway activation by immune complexes in SLE and is the last event leading to tissue damage in many organs.[34, 35] The rs10818488A>G variant, located in an intergenic region between *C5* and *TRAF1*, generates a binding site to histone acetyltransferase EP300 that regulates transcription via chromatin remodeling, and that may be involved in *TRAF1* and/or *C5* regulation.[36] The rs3761847G>A variant, located in the *TRAF1* 5′ intronic region, seems to be associated to low levels of TRAF1 mRNA and protein, and increased production of proinflammatory cytokines due to negative regulation of Toll-like receptor signaling, contributing to the increased incidence and severity of inflammatory diseases.[37]

Considering the role of *PTPN22* and *TRAF1/C5*, as well as the prevalence of SLE in the female population and the genetic heterogeneity among Mexican mestizo populations from geographically distant regions, our objective was to analyze whether the *PTPN22* rs2488457C>G and *TRAF1-C5* rs10818488A>G and rs3761847G>A variants are associated with the susceptibility to develop SLE in women from Yucatán where the Amerindian contribution is mainly of Mayan ancestry and from Central Mexico with different ethnical groups.[38]

Materials and Methods.

ETHICS STATEMENT.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics, Biosecurity and Research committees of General Hospital in Merida, Yucatan (CIE: 008-1-11), and the Juárez Hospital of Mexico, Mexico City (HJM: 0446/18-I), respectively. The confidentiality of the participants was strictly maintained.

STUDY POPULATION.

An observational and cross-sectional study was conducted using DNA samples stored at -20°C by 4 years, from both SLE patients and controls of Yucatan and Central Mexico. DNA was isolated from peripheral blood leukocytes by a slightly modified version of the standard method, involving proteinase K digestion and salification.[39] The quality and quantity of the genetic material was determined using a Thermo Scientific NanoDrop One/One spectrophotometer. All DNA samples from cases and controls were brought to a final concentration of 10 ng/µl for the genotyping assays.

We selected 100 samples of SLE patients and 100 control subjects, all of them women of Mayan ancestry, with the inclusion criteria as people born in Yucatan with a surname derived from Spanish, with Mexican Mayan ancestors at least up to the third generation, and at least one parent and their direct ascendent also born in Yucatan. Mayan women were selected using anthropological and

demographic parameters such as language, place of birth, surnames, genealogy, and lifestyle history to match all cases and controls ethnically. We also analyzed 100 samples of SLE patients and 100 controls subjects, all women from the Central States of the country including Mexico City, belonging to different ethnic groups, which were provided by the Rheumatology Service, Hospital Juárez of México, Mexico City. Patients were diagnosed by a Rheumatologist, according to the criteria of the American College of Rheumatology,[40] who signed the informed consent letters to participate in the study. Controls were individuals with no family history of autoimmune or inflammatory diseases such as obesity, type 2 diabetes, cardiovascular disease, among others. The sample size was calculated using the Quanto V1.2 program, considering the prevalence of SLE in Mexico,[9, 10] and the minor allelic frequencies of variants of each gene, reported in the 1000 Genomes Project.

SNV GENOTYPING.

From the DNA samples (10 ng), *PTPN22* rs2488457C>G and *TRAF1-C5* rs10818488A>G and rs3761847G>A, were analyzed by allelic discrimination assays using TaqMan probes (rs2488457: C_16027865_10; rs10818488: C__2783655_10; rs3761847: C__2783640_10), following supplier's specifications (Thermo Fisher Scientific®). The amplification was carried out in the StepOne real-time PCR thermocycler (Applied Biosystems®), according to the following conditions: a first incubation at 50°C for 2min, followed by the activation of the polymerase at 95°C for 10min, and 40 cycles of 95°C for 15s, 60°C for 1min and 60°C for 30s. To validate the assays, two wells without genetic material were included as negative controls. The results were interpreted according to the fluorescence signals of the labeled probe and considering the ROX reference marker to normalize the fluorescence variations. Allelic and genotypic frequencies were determined using data obtained from the amplification products generated using the StepOne™ Software V2.3 program.

STATISTICAL ANALYSIS.

Hardy-Weinberg equilibrium and allelic and genotypic frequencies were found using the SNPStat.[41] The SNVs association and susceptibility risk analysis was performed by comparing genotypic and allelic frequencies between cases and controls, using the STATA software version 10.2. The haplotypes and linkage disequilibrium (LD) formed by *TRAF1-C5* were analyzed using the Haploview V4.2 software. Genotypic and allelic frequencies of the Yucatecan population were compared with those of Central Mexico, using the chi-square test (x^2) and contingency tables, considering significant values of $p < 0.05$. Multivariate multidimensional scaling (MDS)[42] analysis was performed, using the Arlequin 3.1 program and the Bonferroni correction, to obtain significant genetic distance (F_{ST} value) and p values <0.005, comparing the *TRAF1-C5* rs10818488G>A genotypes of our populations with those of Turkey,[29] Japan,[43, 44] Greece,[45] Colombia,[46] Egypt,[47] Spain and Netherlands.[26] Subsequently, using the "SPSS 2.0" statistical package, the genetic distance values were used to obtain the MDS of SLE cases.

Results.

It is the first study comparing female cases with SLE and controls, from two geographically distant Mexican populations, the Yucatecan with a predominant Mayan ancestral component, and the one from Central Mexico with different ethnic origins. The mean age of cases with SLE from both populations was not significantly different (39 ± 13 vs 38 ± 11, $p = 0.557$), but it was significantly lower in the controls of the Yucatecan population compared to those from Central Mexico, respectively (37 ± 13 vs 52 ± 4, $p < 0.0001$). The statistical power of our study was proved with the Quanto V1.2 program, using the frequency of the risk allele of the respective variants, the prevalence of SLE in both populations and the size of the selected sample, which was sufficient to reach 93 % and 89% power, respectively.

The *PTPN2* and *TRAF1-C5* variants were in Hardy-Weinberg equilibrium in both case and control populations ($p > 0.05$). Distribution of *PTPN22 rs2488457C>G* revealed a lower frequency of G risk allele ranging from 36% to 48%, being the heterozygous genotype C/G the most frequent in all studied groups. *TRAF1-C5* risk alleles rs10818488A and rs3761847G ranged from 34% to 40%, being the non-risk alleles G and A the most frequent in all studied groups, whereas the heterozygous genotypes were the most frequent in cases of Yucatan and controls from Central Mexico, as well as the homozygous genotype was the most frequent in controls from Yucatan and cases from Central Mexico for both *TRAF1-C5* variants. Distribution of allelic and genotype frequencies of *PTPN22* or *TRAF1-C5* variants did not show significant difference ($p > 0.05$) between SLE cases and controls for any studied population, suggesting a lack of genetic association of *PTPN22* or *TRAF1-C5* variants for the risk to SLE in Yucatán and Central Mexico (**Table 1**).

Table 1. Allelic and genotypes frequency of *PTPN22* rs2488457C>G and *TRAF1-C5* rs10818488A>G and rs3761847G>A variants in association with SLE in Yucatan and Central Mexico.

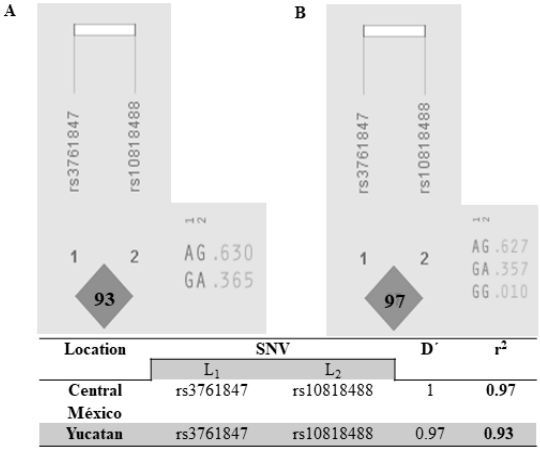
PTPN22	**Yucatan**				**Central Mexico**			
rs2488457	**Cases n (%)**	**Controls n (%)**	***p***	**OR (IC 95%)**	**Cases n (%)**	**Controls n (%)**	***p***	**OR (IC 95%)**
C	119 (60)	105 (52)		Reference	110 (55)	127 (64)	Reference	
G	81 (40)	95 (48)	0.20	0.75 (0.50-1.11)	90 (45)	73 (36)	0.12	1.38 (0.92-2.06)
C/C	37 (37)	32 (32)		Reference	31 (31)	38 (38)	Reference	
C/G	45 (45)	41 (41)	0.87	0.95 (0.50-1.79)	48 (48)	51 (51)	0.65	1.15 (0.62-2.14)
G/G	18 (18)	27 (27)	0.15	0.58 (0.27-1.23)	21 (21)	11 (11)	0.05	2.34 (0.98-5.58)
TRAF1-C5								
rs10818488								
G	122 (61)	133 (66)		Reference	129 (64)	123 (62)	Reference	
A	78 (39)	67 (34)	0.25	1.27 (0.84-1.91)	71 (36)	77 (38)	0.53	0.88 (0.59-1.32)
G/G	35 (35)	47 (47)		Reference	44 (44)	38 (38)	Reference	
G/A	52 (52)	39 (39)	0.06	1.79 (0.98-3.27)	41 (41)	47 (47)	0.36	0.75 (0.41-1.38)
A/A	13 (13)	14 (14)	0.62	1.25 (0.52-2.98)	15 (15)	15 (15)	0.73	0.86 (0.37-1.99)
rs3761847								
A	120 (60)	133 (66)		Reference	129 (64)	125 (62)	Reference	
G	80 (40)	67 (34)	0.18	1.32 (0.88-1.99)	71 (36)	75 (38)	0.68	0.92 (0.61-1.38)
A/A	35 (35)	48 (48)		Referencia	44 (44)	40 (40)	Referencia	
A/G	50 (50)	37 (37)	0.06	1.85 (1.00-3.41)	41 (41)	45 (45)	0.64	0.82 (0.45-1.51)
G/G	15 (15)	15 (15)	0.52	1.37 (0.59-3.17)	15 (15)	15 (15)	0.83	0.91 (0.39-2.09)

TRAF1-C5 rs10818488A>G and rs3761847G>A variants generated three and four haplotypes in Central Mexico and Yucatan populations, respectively (**Table 2**). The AG haplotype was the most frequent in all studied groups of both Central Mexico and Yucatan populations. Distribution of frequencies for *TRAF1-C5* haplotypes did not show significant differences between SLE cases and their controls, suggesting no association of *TRAF1-C5* haplotypes with the genetic risk for developing SLE. Comparison of frequencies of *TRAF1-C5* haplotypes neither was significantly different between Central Mexico and Yucatan populations.

Table 2. Analysis of *TRAF1-C5* rs3761847G>A and rs10818488A>G haplotypes associated with SLE in Yucatan and Central Mexico.

	Haplotypes		**Cases**		**Controls**		***p***	**OR (IC 95%)**
	rs3761847	**rs10818488**	**2n**	**%**	**2n**	**%**		
Central Mexico	A	G	130	65	122	61	0.46	1.18 (0.79-1.78)
	G	A	70	35	76	38	0.60	0.87 (0.58-1.32)
	A	A	0	0	2	1	0.5	0
Yucatan	A	G	120	60	130	65	0.35	0-80 (0.53-1.21)
	G	A	78	39	64	32	0.17	1.35 (0.90-2.04)
	G	G	2	1	2	1	1	1 (0.13-7.16)
	A	A	0	0	2	1	0.5	0

The association study of *TRAF1-C5* with the disease was extended through Linkage Disequilibrium (LD) analysis, considering the location and proximity of the polymorphic variants in the gene. Two haplotypes are seen for the population of Central Mexico and three for Yucatan. The AG haplotype occurs more often, and with strong DL in the Yucatecan and Central Mexican cases with significant correlation values ($r^2 > 0.93$) associated with the rs3761847 and rs10818488 variants (**Figure 1**).



Location	SNV		D´	r^2
	L_1	L_2		
Central México	rs3761847	rs10818488	1	**0.97**
Yucatan	rs3761847	rs10818488	0.97	**0.93**

Figure 1. Linkage disequilibrium analysis of *TRAF1-C5* rs10818488G>A and rs3761847A>G variants between cases (n=100) vs. controls (n=100) from Central Mexico (A) and cases (n=100) vs. controls (n=100) from Yucatan (B).

To find differences between our both two Mexican populations, allelic and genotypic frequencies of the *PTPN22* rs2488457C>G and *TRAF1-C5* rs10818488G>A and rs3761847A>G SNVs were compared between cases and controls. No significant difference was seen for any group ($p > 0.05$), except in controls for both allelic ($p = 0.02$) and genotype ($p = 0.01$) distribution of *PTPN22* rs2488457C>G (**Table 3**), suggesting differences among healthy women from Yucatan and Central Mexico, due to ethnical variations of *PTPN22* rs2488457C>G among populations.

Table 3. Comparison of allelic and genotypic frequencies of risk alleles of *PTPN22* and *TRAF1-C5* variants between cases and controls from Yucatecan and Central Mexico.

Variants	Allele and genotype	Cases Yucatan vs Central Mexico *p*	Controls Yucatan vs Central Mexico *p*
PTPN22			
rs2488457	C	0.36	0.02
	G		
	C/C	0.65	0.01
	C/G		
	G/G		
TRAF1-C5			
rs10818488	G	0.46	0.29
	A		
	G/G	0.29	0.42
	G/A		
	A/A		
rs3761847	A	0.35	0.40
	G		
	A/A	0.38	0.47
	A/G		
	G/G		

Furthermore, interpopulation genetic variability was assessed using several populations reported in the literature, where the available *TRAF1-C5* rs10818488G>A genotypes from each population were extracted, and MDS was used to plot F_{ST} value. To show differences or similarities between our populations with those of Turkey, Spain, Greece, Netherland, Egypt, Japan, and Colombia, the MDS analysis was conducted using the *TRAF1-C5* rs10818488G>A genotypes from each

population. By the "Arlequin" statistical program and the Bonferroni correction, genetic distance and $p < 0.005$ values were determined and used in the "SPSS 2.0" statistical package to obtain the MDS of the cases. Data shows that European populations are positioned around the center of the MDS plot, although only Turkey, Spain, and Greece (**Figure 2**), which might share a common element of susceptibility to SLE, have previously been associated with the disease. In addition, Latin American (LA) admixed groups have arranged in a cluster, surrounded by Europeans, possibly because of the important contribution of Caucasian ancestry to the genetic pool of the LA population. Finally, we saw significant differences in most of the populations when compared with Japanese ($p < 0.005$; bold diamond in **Figure 2**), which has an Asian ancestry; conversely, Egypt, the African group, did not present differences with any group and was found near LA communities (**Table 4**).

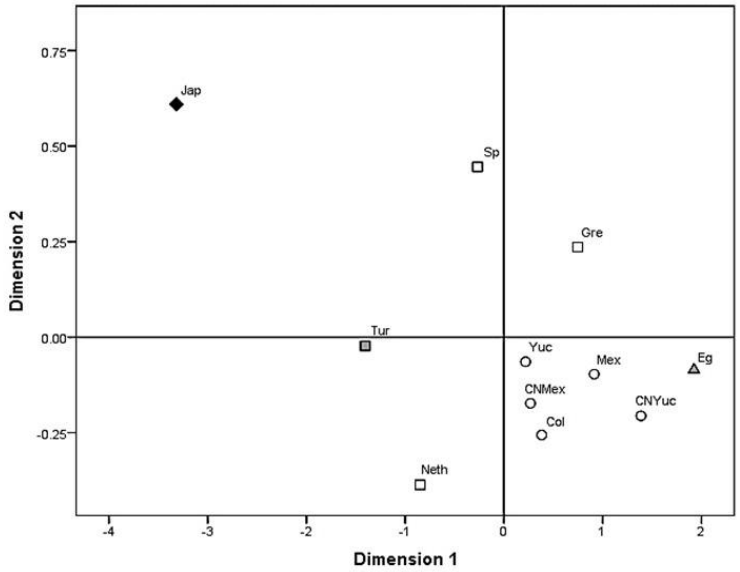


Figure 2. MDS analysis of *TRAF1-C5* rs10818488G>A genotypes in cases with SLE from different populations; Yuc: Yucatan, Mex: Central Mexico, Tur: Turkey, Sp: Spain, Neth: Netherland, Gre: Greece, Jap: Japan, Col: Colombia, Eg: Egypt, CNYuc: Controls from Yucatan, CNMex: controls from Central Mexico.

Table 4. Significant *p* values (below the * diagonal) of F_{ST} according to *TRAF1-C5* rs10818488G>A genotypes.

	Yuc	Mex	Tur	Sp	Neth	Gre	Jap	Col	Eg	CYuc	CMex
Yuc	*										
Mex	0.5314	*									
Tur	0.1480	0.0307	*								
Sp	0.6403	0.1574	0.1380	*							
Neth	0.3112	0.0824	0.6311	0.3977	*						
Gre	0.6084	0.7943	0.0110	0.1061	0.0524	*					
Jap	**0.0002**	**0.0000**	0.0186	**0.0000**	**0.0022**	**0.0000**	*				
Col	0.8540	0.6407	0.0640	0.3710	0.1801	0.8133	**0.0001**	*			
Eg	0.1589	0.4529	**0.0037**	0.0218	0.0104	0.2461	**0.0000**	0.1901	*		
CYuc	0.3079	0.7494	0.0084	0.0507	0.0304	0.4391	**0.0000**	0.3718	0.7427	*	
CMex	0.9999	0.5992	0.1237	0.5364	0.2731	0.6672	**0.0001**	0.9229	0.1998	0.3466	*

Significant *p* values of F_{ST} according to *TRAF1-C5* rs10818488G>A genotypes. Yuc: Yucatan, Mex: Central Mexico, Tur: Turkey, Sp: Spain, Neth: Netherland, Gre: Greece, Jap: Japan, Col: Colombia, Eg: Egypt, CYuc: Controls from Yucatan, CMex: controls from Central Mexico.

Discussion.

PTPN22 represents the second loci strongly associated with SLE,[48,49] and encodes the Lyp protein, which plays a key role in regulating the activation and effector functions of T and B cells, neutrophils, dendritic cells, and monocytes.[50-54] *PTPN22* has strong evidence of association with SLE,[55] and other ADs such as T1D,[19] RA,[22, 56] and Graves' disease.[57]

R620W is the main *PTPN22* variant associated with the different ADs. However, others such as rs2488457C>G, have been scarcely studied and their role in susceptibility to SLE is not clear. Thus, we evaluated this variant in cases and controls from Yucatán and Central Mexico. After analysis, we did not find an association of *PTPN22* rs2488457 with SLE in the cases of Yucatan and Central Mexico (**Table 1**). These agree with those reported by Machado *et al.*,[25] in western Mexico, who analyzed rs2476601 and rs2488457, and saw that the allele (C) and genotype (C/C) frequencies did not show an association with the disease. Furthermore, despite the strong DL they presented, they did not observe an association with the disease when evaluating the haplotype formed by the variants. The similarity in the results could be due to the closeness of the western populations with that of Central Mexico, and the heterogeneity between these and the Yucatecan population.

The rs2476601 variant has been the focus of many research studies. In this regard, Moez *et al.*,[47] analyzed this variant in Egyptian patients, where the heterozygous genotype C/T occurred more often in cases and was associated with the development of the disease, while the risk homozygous T /T was found to be absent. Additionally, they studied the correlation of variants with clinical manifestations such as joint and renal involvement. They found that patients with renal involvement have a higher frequency of the C/T heterozygote. On the other hand, Kariuki *et al.*,[58] reported the T allele associated with low IFN-γ and high IFN-α levels in SLE patients. Unfortunately, our study did not analyze rs2476601C>T or determine the relation of allelic and genotypic frequencies of rs2488457C>G with clinical manifestations or IFN-α levels. Still, we showed no association of rs2488457C>G with SLE; however, due to the multifactorial nature of the disease, it is essential to consider other *PNP22* variants in our populations.

Lyp protein is expressed in B cells, monocytes, neutrophils, dendritic cells and natural killer cells.[59] It has been suggested that the risk of developing SLE could be due to alterations in the function of dendritic cells that express low levels of Lyp, allowing overactivation of T cells and a decrease in the regulatory T cells.[60] The rs2488457C>G plays a crucial role in decreased Lyp expression leading to the continued activation of autoreactive T and B cells.[61] In addition, Machado *et al.*,[25] found that Lyp mRNA levels were 2.8 times lower in patients with severe SLE compared to inactive and control subjects. The *PTPN22* mRNA expression did not show differences among genotypes suggesting that their absence or decrease contributes to the hyperactivity of T and B cells and disease pathogenesis. On the other hand, not only the expression of Lyp could be affected in patients, but also the isoforms of the protein, such as Lyp2 and Lyp22.2.[62] Clinical importance of these isoforms and their relevance in the pathogenesis of SLE have yet to be found.

Regarding the *TRAF1-C5* rs10818488 and rs3761847 variants, we found no association with the development of SLE, and there are no reports in other Mexican populations. Our results contrast with what was reported by Zervou *et al.*,[29] who analyzed rs10818488G>A in the Turkish people and observed that the A allele and A/A genotype were more frequent in cases compared to controls (18.3%, 47.7% vs 12.2%, 36.4%), suggesting its role as a genetic risk factor for SLE (OR=2.17, CI=1.08-4.35, $p = 0.002$). Similarly, Kurreeman *et al.*,[26] suggested an association of the Spanish and Greece population with the disease; however, this was not seen in the Netherlands. Coincidently, Nishimoto *et al.*,[44] saw that the A allele of rs10818488G>A did not show an association with SLE in the Japanese population (OR=1.06, CI=0.93- 1.20, $p = 0.43$). Furthermore, Palomino *et al.*,[46] did not find this association in the Colombian population (OR=0.88, IC=0.58-1.32, $p = 0.5$). Likewise, Nishimoto *et al.*,[44] analyzed the rs3761847G>A in the Japanese population and saw no association with the disease (OR=1.08, CI=0.95-1.23, $p = 0.28$).

All these data suggest that the contribution of *TRAF1-C5* to the development of SLE is different between the populations where the ancestral component seems to play a key role in the development of the disease.[63] For example, Xu *et al.*,[43] in an earlier meta-analysis with European, Asian, and Colombian populations, the same ones used in Table 4, did not reveal a significant overall association with SLE; however, upon further stratification by ethnicity, they discovered that an allelic and genotypic association was preserved only for Europeans. Similarly, Hernandez *et al.*,[64] through an admixture analysis with HLA genes, saw

a greater load of European ancestry in Mexican SLE patients. The origin of risk alleles and HLA haplotypes were from southwestern Europe, while protection alleles came from Mexican Native Americans. In addition, they claimed that the susceptibility to SLE could vary throughout Mexico as the ethnic pattern does, and where the European risk component would have a significant role in its appearance. Regarding this, several reports have outlined the genetic substructure of Mexican mestizos, with a consensus about the impact of Amerindian, Caucasian, and African ancestry throughout Mexico; an increase of the Amerindian component to the south of Mexico and, conversely, an increase in the Caucasian ancestry to the north.[65, 66] Even more, the absence of significant differences between Mexican SLE patients and those of Turkey, Spain, and Greece (Table 4), where a significative association with the disease has been proved, seems to contradict the impact of Caucasian ancestry. However, the lack of susceptibility of LA populations may be attributed to ethnic fluctuations, whereby the impact of Caucasian lineage is diluted by the influence of Amerindian and African ancestries in Mexico,[38, 66] and Colombia,[67] respectively. In fact, Hernandez *et al.*,[64] argue that SLE's severity and clinical manifestations differed in an ethnic-dependent manner even when susceptibility HLA haplotypes were shared between Mexican populations. Finally, although the analysis was based on a single genetic variant, the assumptions about the ancestry's influence on SLE risk [63] are consistent with the MDS plot based on pairwise F_{ST} genetic distances (**Figure 2**).

Due to the few studies on *TRAF1-C5* rs10818488G>A and rs3761847A>G we evaluated their haplotypes. The analysis did not show an association with the development of SLE in our populations; however, we observed a strong LD, suggesting that they are co-inherited together. Although the haplotypes are in DL, it is unclear whether the risk alleles or the group of alleles influence *TRAF1* and/or *C5* to increase the susceptibility of this disease, or if these alleles may exert their effect through a neighboring gene.[68] The strong LD saw can be attributed to the rapid growth of genetically distant populations, which determine large regions of allelic association throughout the genome, and to natural selection, whereby certain alleles are increased or decreased due to population mixing. Presenting a strong DL suggests that the haplotype is inherited from one generation to the next as individual units.[69]

The intermixing of European and Amerindian populations in Yucatan and Central Mexico unequivocally emphasizes the critical role that the ancestral component plays in the development of SLE. On the other hand, the importance of *PTPN22* and *TRAF1/C5* in the inflammatory process and proliferation of T and B cells, suggest they might be acting with other genes for susceptibility of this disease, but further studies are needed to confirm this assessment in the Mexican population.

Conclusion

The *PTPN22 rs2488457C>G and TRAF1-C5 rs10818488A>G and rs3761847G>A* variants, as well as the *TRAF1-C5* haplotypes, are not associated with the development of SLE. However, the strong DL observed in the *TRAF1-C5* haplotypes suggests that they are inherited together from one generation to the next and could be involved in the development of the disease in association with other genes or risk factors. Likewise, a clear Caucasian influence was seen with the analysis of *TRAF1-C5* rs10818488A>G, although the susceptibility to SLE would have been changed by the influence of non-Caucasian components.

Conflicts of interest statement

The authors have no conflicts of interest to declare.

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Data availability statement

The data used to support this study's findings are available from the corresponding author upon request.

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