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BLK and *BANK1* polymorphisms and interactions are associated in Mexican patients with systemic lupus erythematosus

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Abstract

Objectives The *BLK* and *BANK1* genes have been consistently associated with systemic lupus erythematosus (SLE), primarily in European or Asian-derived populations. However, this finding has not been replicated in Latin-American patients.
Methods Our study included 881 women from Mexico: 487 healthy controls and 394 SLE patients. The *BLK* rs13277113A/G-rs2736340T/C as well as *BANK1* rs10516487G/A (R61H)-rs3733197G/A (A383T) single nucleotide polymorphisms (SNPs) were evaluated using a TaqMan® SNP genotyping assay.
Results Our data showed that the *BLK* rs2736340T/C and rs13277113A/G polymorphisms are associated with susceptibility to SLE (C vs T, OR 1.60, $p=2\times10^{-5}$; G vs A, OR 1.53, $p=9\times10^{-5}$, respectively). We also identified an association between the functional *BANK1* R61H polymorphism and SLE (A *vs* G, OR 1.56, $p=0.002$). In addition, we observed a genetic interaction between *BLK* (rs2736340T/C, rs13277113A/G) and *BANK1* (R61H and A383T) associated with susceptibility to SLE.
Conclusion This is the first study documenting an association between *BLK* and *BANK1* and SLE in a Latin-American population. Our data confirm previous reports: *BLK* and *BANK1* are factors associated with SLE. Thus, both genes are universal *loci* for this autoimmune disease.

Keywords Systemic lupus erythematosus · Single nucleotide polymorphisms · *BLK* · *BANK1* · Association · Susceptibility

Introduction

The *BLK* (B-lymphoid tyrosine kinase) gene encodes a non-receptor tyrosine kinase of the src family that is involved in B-cell receptor signaling and development [1, 2]. It has been shown that BLK, similar to other members of the src family of protein tyrosine kinases (such as Lyn), interacts with BANK1, an adaptor/scaffold protein primarily expressed in B cells [3]. In addition, BANK1 has an important role in B-cell activation [4]. Thus, both proteins play an essential role in B-cell signaling and activation. Recently, various genome-wide association studies (GWAS) identified different single nucleotide polymorphisms (SNPs) in *BLK* (rs13277113A/G and rs2736340T/C) and *BANK1* (rs10516487C/T R61H and rs3733197G/A A383T) to be associated with systemic lupus erythematosus (SLE), primarily in European and Asian-derived populations [4–9]. Furthermore, different gene candidate studies have consistently replicated these findings [10–22]. However, the association of these four *BLK* and *BANK1* SNPs and SLE has been scarcely evaluated in Latin-American populations. Thus,

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our aim was to determine whether the *BLK* rs13277113A/G and rs2736340T/C as well as *BANK1* R61H and A353T polymorphisms are associated with SLE or lupus nephritis (LN) in a Mexican population. Further, we looked for a genetic interaction between *BANK1* and *BLK* in our study population.

Materials and methods

Patients

This study included 487 healthy controls and 394 patients with SLE from Central Mexico. Cases and controls were unrelated women over 18 years old and of self-reported Mexican-Mestizo ancestry (for three generations). SLE patients with concomitant autoimmune diseases (ADs), cancer and genetic syndromes were excluded. Individuals with no family history of autoimmune or chronic inflammatory disease (including obesity, asthma, food allergy, inflammatory bowel disease, chronic urticarial, and others) were recruited as controls (these characteristics have previously been reported [23]). Demographic characteristics of patients with SLE and controls are shown in Table 1. SLE patients were classified according to the 1997 American College of Rheumatology criteria [24]. LN data were available for 276 SLE patients, 105/276 presented LN. The patients were recruited from the rheumatology services of Hospital Juárez de México (HJM) and Centro Médico Nacional "La Raza", Instituto Mexicano del Seguro Social, respectively; meanwhile, the controls were obtained from the blood bank of HJM. Our protocol was conducted in compliance with the Declaration of Helsinki and was approved by the Biosecurity, Ethics and Research committees of HJM (HJM 0446/18-I).

DNA extraction

From each patient and control, we obtained a total of 5–8 mL of EDTA-treated peripheral blood. Nuclear DNA was isolated from leukocytes with the Invisorb® Blood Universal Kit according to the manufacturer's specifications. Briefly, the leukocytes were lysed and proteins were removed by protein digestion. Next, DNA was precipitated, washed, and resuspended in nuclease-free water and stored until needed (User Manual Invisorb® Blood Universal Kit Stratec Molecular GmbH, D-13125 Berlin, Germany, 2018).

Table 1 Demographic distribution in healthy individuals and in patients with SLE

	Controls *n* (%)	SLE *n* (%)
Total	487 (100)	394 (100)
Age (SD)	52.7 ± 7.9	38 ± 12
LN/non-LN	–	105 (38)/171 (62)
ANA ±	–	198 (90.8)/20 (8.8)

SLE systemic lupus erythematosus, *SD* standard deviation, *LN* lupus nephritis, *ANA* antinuclear antibodies

Genotyping

The *BLK* and *BANK1* genotypes were obtained with a TaqMan® SNP genotyping assay according to the manufacturer's instructions (ThermoFisher, Foster City, CA, USA). Each PCR included 10 ng of DNA, 2.5 μL of TaqMan Master Mix, 0.0625 μL of probe (40X) and 2.435 μL of DNase-free water in a final volume of 5.0 μL. The PCR protocol included denaturing at 95 °C for 10 min, followed by 45 cycles of denaturing at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. To determine the genotypes, we used the Bio-Rad CFX Manager 3.1 software implemented in the CFX96 Touch TM Real-Time PCR system (Bio-Rad, California, USA). Two researchers determined the genotype distribution in the discrimination allelic plot. We genotyped all samples twice (both cases and controls) and observed a reproducibility of 100%.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) of each *BLK* and *BANK1* SNP was calculated using Finetti software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). A p value < 0.05 in controls was indicative of deviation from HWE. Association among the alleles/genotypes and the disease was analyzed by comparing cases and controls. We applied the Bonferroni correction test to all our data (p value ≤ 0.05 indicated an association). The *BLK* and *BANK1* SNPs were evaluated under the allelic, codominant, dominant, and recessive genetic models using the Epidat 3.1 software (http://www.sergas.es/MostrarContidos_N3_T01.aspx?IdPaxina=62715). Haploview software was used for the haplotype analysis and to determine the linkage disequilibrium (LD) among *BLK* and *BANK1* markers [25]. The multifactor dimensionality reduction (MDR) program (V 3.0.2) was used to evaluate SNP–SNP interactions [26]. Quanto software was used to calculate the statistical power of our study (http://biostats.usc.edu/cgi-bin/DownloadQuanto.pl).

Results

HWE and statistical power

Genotypic distribution of the *BLK* rs13277113A/G ($p = 0.67$), rs2736340T/C ($p = 0.60$) and *BANK1* rs10516487G/A ($p = 0.39$) SNPs in controls was in HWE;

however, we identified a weak deviation from HWE in *BANK1* rs3733197G/A ($p=0.02$). The statistical power of our study was 94.7% (under a recessive model, and taking into account the minor allele frequency of *BANK1* rs10516487A).

Association analysis of *BLK* and *BANK1* with SLE

The genotype and allelic frequencies of *BLK* rs2736340T/C were different in cases compared with controls. Our data showed that using the allelic, codominant or recessive models and taking into account the major allele or major genotypes, this polymorphism was associated with susceptibility to SLE (Table 2). Under the recessive model, we observed a strong statistical significance (OR 1.85, $p=7\times10^{-6}$) even after we applied the Bonferroni correction test ($p=2.8\times10^{-5}$). Similar results were identified with *BLK* rs13277113A/G (Table 2). The *BANK1* rs10516487G (R61) major allele also showed an association with susceptibility to SLE, A vs G, OR 1.56, $p=0.002$ (Table 3), which remained after the Bonferroni correction test ($p=0.008$). On the other hand, the *BANK1* rs3733197 (A353T) GG (AA vs GG) or GA (AA vs AG) genotypes showed no association with SLE after the Bonferroni test (although a trend towards an association with susceptibility was observed) (Table 3). The analysis between the *BLK* and *BANK1* SNPs did not show an association with LN (data not shown).

Genetic interactions among *BLK* and *BANK1* SNPs in SLE

To determine whether a genetic interaction between the four *BLK* and *BANK1* SNPs was associated with SLE, we carried out a comparison of the distribution of genotypes in cases and controls (Fig. 1). Our data revealed different interactions between *BLK* and *BANK1* genotypes. Thus, the association previously identified with susceptibility to SLE is best explained by *BLK* rs13277113A/G-rs2736340T/C and *BANK1* rs10516487G/A-rs3733197 interactions. Data obtained by MDR showed that the best interaction model was *BLK* rs2736340T/C-rs13277113A/G- and *BANK1* rs10516487G/A-rs3733197G/A (testing accuracy = 0.5678 and cross-validation consistency = 10/10), which also showed a significant association with susceptibility to SLE (OR 2.22, $p<0.0001$) (Table 4). Additionally, we also identified other genetic interactions between *BLK* and *BANK1* in our study population (Table 4). Dendrogram analysis showed that the *BLK* rs2736340T/C and rs132777133A/G SNPs

Table 2 Genotypic and allelic frequencies of the *BLK* polymorphisms and association analysis in patients with SLE and healthy individuals

SNP	Model	Genotypes or alleles	SLE *n* (%)	Controls *n* (%)	OR 95% CI	*p**	*pc***
BLK rs13277113	Codominant	AA	238 (60.4)	226 (46.4)	1.86 (1.11–3.12)	0.017	NS
		AG	130 (33.0)	215 (44.2)	1.07 (0.63–1.81)	NS	NS
		GG	26 (6.6)	46 (9.4)	–	–	–
		A	606 (76.9)	667 (68.5)	1.53 (1.24–1.90)	0.00009	0.00036
		G	182 (23.1)	307 (31.5)	–	–	–
	Dominant	AA + AG	368 (93.4)	441 (90.6)	1.48 (0.89–2.43)	NS	NS
		GG	26 (6.6)	46 (9.4)	–	–	–
	Recessive	AA	238 (60.4)	226 (46.4)	1.76 (1.35–2.31)	0.000002	0.000008
		GG + GA	156 (39.6)	261 (53.6)	–	–	–
BLK rs2736340	Codominant	TT	242 (61.4)	225 (46.2)	1.98 (1.18–3.33)	0.009	0.036
		TC	127 (32.2)	216 (44.4)	1.08 (0.63–1.85)	NS	NS
		CC	25 (6.4)	46 (9.4)	–	–	–
		T	611 (77.5)	666 (68.4)	1.60 (1.29–1.98)	0.00002	0.00008
		C	177 (22.5)	308 (31.6)	–	–	–
	Dominant	TT + TC	369 (93.7)	441 (90.6)	1.54 (0.93–2.55)	0.09	NS
		CC	25 (6.3)	46 (9.4)	–	–	–
	Recessive	TT	242 (61.4)	225 (46.2)	1.85 (1.42–2.43)	0.000007	0.000028
		CC + CT	152 (38.6)	262 (53.8)	–	–	–

Taking into account the *BLK* rs13277113G (and BLK rs2736340C) minor allele we identify the following: A vs G: OR 0.65, $p=0.00009$; AA vs AG: OR 0.57, $p=0.0001$; AA vs GG: OR 0.54, $p=0.017$; AA vs AG + GG: OR 0.57, $p=0.00004$; AA + AG vs GG: NS. Regarding the *BLK* rs13277113G, we identify the following: T vs C: OR 0.63, $p=0.00002$; TT vs TC: OR 0.55, $p=0.00003$; TT vs CC: OR 0.51, $p=0.009$; TT vs TC + CC: OR 0.54, $p=0.0000067$

SNP single nucleotide polymorphism, *OR* odds ratio, *CI* confidence interval, *SLE* systemic lupus erythematosus, *NS* not significant

*$p<0.05$, statistically significant. ***pc*: *p*-corrected after Bonferroni test

Table 3 Genotypic and allelic frequencies of the *BANK1* polymorphisms and association analysis in patients with SLE and healthy individuals

SNP	Model	Genotypes or alleles	SLE *n* (%)	Controls *n* (%)	OR 95% CI	*p**	*pc***
BANK1	Codominant	GG	311 (78.9)	347 (71.2)	6.72 (1.52–29.63)	0.004	0.016
rs10516487		GA	81 (20.6)	125 (25.7)	4.86 (1.08–21.82)	0.024	NS
R61H		AA	2 (0.5)	15 (3.1)		–	–
		G	703 (89.2)	819 (84.1)	1.56 (1.18–2.08)	0.002	0.008
		A	85 (10.8)	155 (15.9)	–	–	–
	Dominant	GG + GA	392 (99.5)	472 (96.9)	6.23 (1.42–27.4)	0.006	0.024
		AA	2 (0.5)	15 (3.1)	–	–	–
	Recessive	GA + AA	311 (78.9)	347 (71.2)	1.51 (1.11–2.06)	0.009	0.036
		GG	83 (21.1)	140 (28.8)	–	–	–
BANK1	Codominant	GG	249 (63.2)	307 (63.0)	2.16 (1.09–4.29)	0.024	NS
rs3733197		GA	133 (33.8)	148 (30.4)	2.40 (1.19–4.84)	0.013	NS
A383T		AA	12 (3.0)	32 (6.6)	–	–	–
		G	631 (80.1)	762 (78.2)	1.12 (0.89–1.41)	0.34	NS
		A	157 (19.9)	212 (21.8)	–	–	–
	Dominant	GG + GA	382 (97.0)	455 (93.4)	2.24 (1.14–4.41)	0.017	NS
		AA	12 (3.0)	32 (6.6)	–	–	–
	Recessive	GA + AA	249 (63.2)	307 (63.0)	1.0 (0.76–1.33)	0.96	NS
		GG	145 (36.8)	180 (37.0)	–	–	–

Taking into account the *BANK1* rs10516487A minor allele we identify the following: G vs A: OR 0.64, $p = 0.002$; GG vs GA: OR 0.72, $p = 0.046$; GG vs AA: OR 0.15, $p = 0.004$; GG vs GA + AA: OR 0.66, $p = 0.009$; GG + GA vs AA: OR 0.16, $p = 0.006$

SNP single nucleotide polymorphism, *OR* odds ratio, *CI* confidence interval, *SLE* systemic lupus erythematosus, *NS* not significant

*$p < 0.05$, statistically significant. ***pc*: *p*-corrected after Bonferroni test

were highly correlated (Both BLK SNPs were in high LD ($r^2 = 0.98$) in cases and controls [Fig. 2]) (Fig. 3). In addition, the *BLK* rs2736340T/C (this variant showed the strongest association with susceptibility to SLE) polymorphism also displayed an interaction with *BANK1* rs10516487G/A (R61H) and rs3733197G/A (T383A) (Fig. 3) or only with *BANK1* rs10516487G/A (R61H), but not with *BANK1* rsrs3733197G/A (Fig. 3 and Table 4); moreover, we did not observe any synergistic interaction among these polymorphisms (Fig. 3).

Haplotypes and LD between *BLK* and *BANK1* polymorphisms in patients with SLE

We identified two haplotypes formed by the combination of the two *BLK* rs2736340T/C and rs13277113A/G polymorphisms (Table 5). The TA haplotype carrying the two major alleles was associated with susceptibility to SLE (OR 1.54, $p = 7.70 \times 10^{-5}$), even after 100,000 permutations ($p = 3 \times 10^{-5}$). Both *BLK* SNPs were in high LD ($r^2 = 0.98$) in cases and controls (Fig. 2a). Otherwise, after the haplotype analysis of *BANK1* rs10516487G/A, and rs3733197G/A, we identified that the AA haplotype carrying the two minor alleles was associated with protection against SLE (OR 0.57, $p = 5 \times 10^{-4}$), even after 100,000 permutations ($p = 0.0015$). *BANK1* SNPs showed low LD ($r^2 < 0.8$) (Fig. 2b). On the other hand, the GA haplotype carrying the rs10516487G major allele showed an association with susceptibility to SLE (OR 1.43, $p = 0.02$) (Table 5); however, this association was lost after correction by 100,000 permutations ($p = 0.07$).

Discussion

SLE—the prototype of ADs—presents a prevalence that varies across the world ranging from 12 to 68 per 100,000 inhabitants [27]. In this way, diverse GWA or candidate gene studies have contributed to the discovery of SLE susceptibility *loci* associated with the prevalence of this AD [5–9]. In addition, several GWAS have reported an association between the *BLK* rs2736340T/C, rs13277113A/G and *BANK1* rs10516487C/T polymorphisms with SLE [4–9]. Additionally, various candidate gene studies carried out in different Asian or European-derived populations have replicated these findings [10–22]. Consistent with these reports, our data also showed an association of *BLK* rs2736340T/C and rs13277113A/G and *BANK1* rs10516487G/A and SLE. Previous studies in European and Asian-derived populations have documented an association between the *BLK* rs2736340T (or rs13277113A) allele and SLE susceptibility

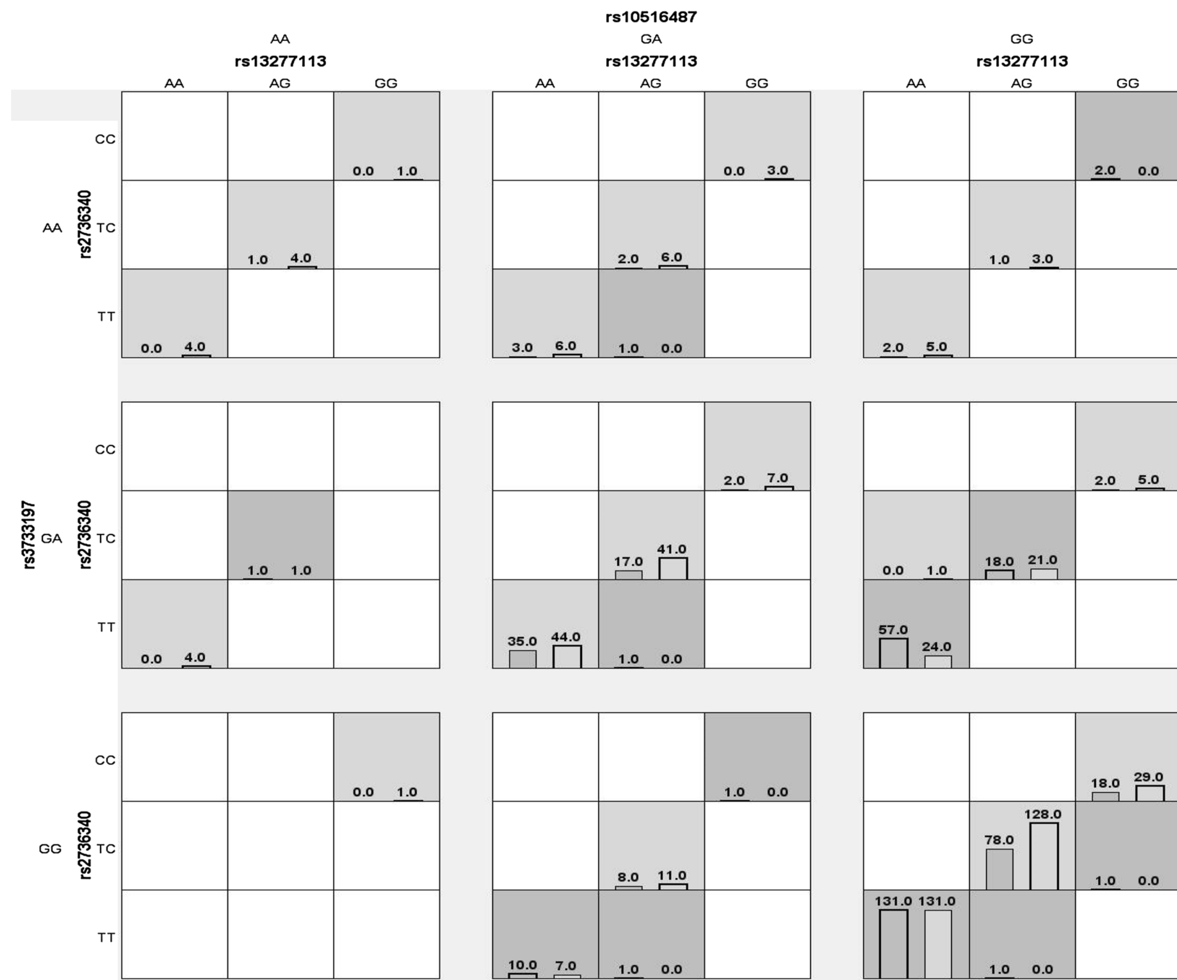

Fig. 1 Distribution of the *BLK* 13277113A/G-rs2736340T/C and *BANK1* rs10516487G/A-rs3733197G/A genotypes in SLE patients and controls. Each cell shows counts of patients with SLE (left) and controls (right). Dark-shaded cells represent "high-risk" genotypes. Light-shaded cells represent "low-risk" genotypes. White-shaded cells represent cases and controls without genotypes

Table 4 Gene–gene interaction models between *BLK* and *BANK1* SNPs in patients with SLE and controls obtained by MDR

Number of factors	Best model[a]	Training accuracy	Testing accuracy	CVC[b]	X^2	*p* value	OR (CI 95%)
1	*BLK* (rs2736340)	0.5761	0.5761	10/10	18.23	< 0.0001	1.85 (1.40–2.46)
2	*BLK* (rs2736340)–*BANK1*(rs10516487)	0.5853	0.5684	7/10	22.80	< 0.0001	1.99 (1.50–2.65)
3	*BLK* (rs2736340)–*BANK1*(rs10516487)–(rs3733197)	0.5948	0.5629	10/10	28.21	< 0.0001	2.16 (1.62–2.87)
4	*BLK* (rs2736340)–(rs13277113)–*BANK1*(rs10516487)–(rs3733197)	0.5981	0.5678	10/10	30.20	< 0.0001	2.22 (1.67–2.95)

*The best model is referred to as the one with the maximum testing accuracy and maximum CVC

**Cross-validation consistency

[5, 10–13, 22, 36] while others have reported that the *BLK* rs2736340C (or rs13277113G) allele is associated with protection against SLE [7, 8, 14–16]. The abovementioned can be explained by the differences among the minor allele frequency, e.g., in European-derived populations, *BLK* rs2736340T is the minor allele [5, 13] while in our populations and those with Asian ancestry, it is the major allele [7, 8, 14]. Of note, the frequency of the *BLK* rs2736340C

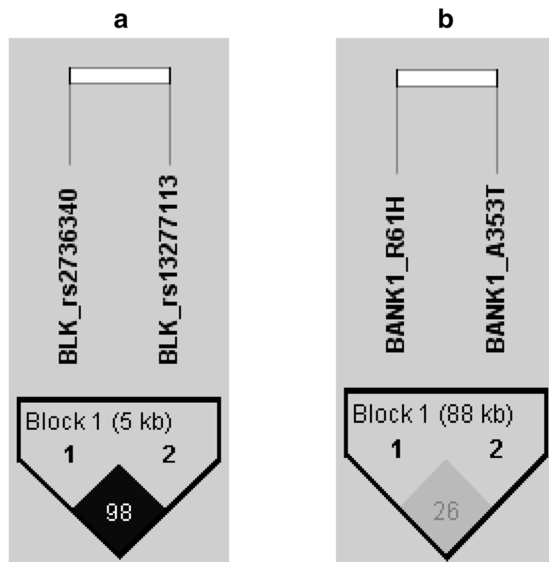

Fig. 2 LD between *BLK* and *BANK1* polymorphisms in patients with SLE and controls. We observed a high LD between the *BLK* rs2736340 and rs13277113 polymorphisms ($r^2 \approx 1$) (1a), mean, both *BANK1* R61H and A383T polymorphisms are not in LD (1b). Thus, both *BANK1* polymorphisms segregate independently

allele reported in the 1000 Genomes Project for Mexicans (Mexican-Americans who live in Los Angeles, CA) (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) is similar to what we found. Our data are in accordance with these studies that report an association with susceptibility to SLE [5, 12, 13, 22]. On the other hand, we also found an association between the *BLK* rs2736340C and rs13277113G minor alleles with protection against SLE under the allelic, codominant and dominant models (data shown at the bottom of Table 2). It is important to note that the *BLK* rs2736340T and rs13277113A major alleles were associated with SLE susceptibility under the allelic, codominant and recessive models, but not under the dominant model.

The TA haplotype carrying the *BLK* rs2736340T and rs13277113A major alleles was also associated with susceptibility to SLE. We identified a high r^2 value (~ 1) for the LD, which means that both SNPs segregate together. The analysis on SniPA server identified different *BLK* SNPs in high LD with rs2736340T/C and rs13277113A/G. Both polymorphisms are TagSNPs for rs2409780, rs2618444, rs2061831, rs2736337, rs2736338, rs1478901, rs9693589, rs2618476, and rs998683 (https://snipa.helmholtz-muenchen.de/snipa/index.php?task=proxy_search), this means that all these *BLK* SNPs could to be associated with SLE. Thus, these *BLK* SNPs (together with rs2736340T/C and rs13277113A/G) may influence SLE susceptibility as haplotypes. In fact, the rs922483 SNP also alters BLK expression [16, 28], and is in high LD with rs2736340T/C and rs13277113A/G (https://snipa.helmholtz-muenchen.de/snipa/index.php?task=proxy_search). As far as we know, our study is the first to report an association between *BLK* and SLE in a Latin-American population.

A functional study showed that the rs13277113A allele is associated with lower levels of BLK mRNA in transformed B-cell lines [5]. Additionally, another study reported that the *BLK* rs2736340T and rs13277113A alleles (as part of a haplotype) associated with risk to SLE affect different biological process in T cells and naïve B cells from healthy individuals and patients with rheumatoid arthritis (RA), such as T-cell proliferation and BCR signaling [29].

BANK1 R61H has been previously associated with SLE in European and Asian-derived populations [4, 7, 9, 10, 13, 14, 17–20]. Our results indicate that the *BANK1* rs10516487A (R61) major allele is associated with susceptibility to SLE, as was previously reported in different populations of Asia and Europe [4, 13, 17, 18]. We also identified an association

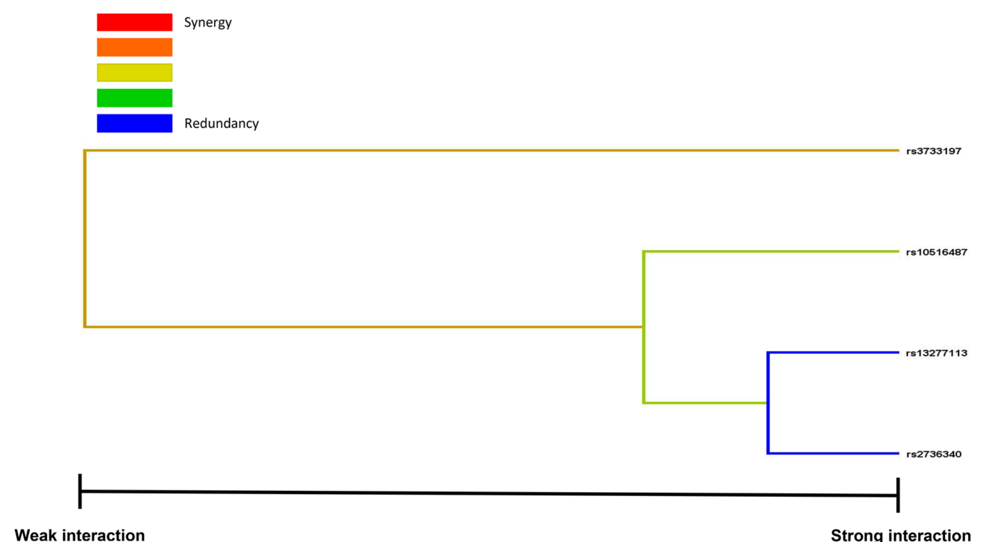

Fig. 3 Dendrogram of interaction between *BLK* (rs13277113A/G and rs2736340T/C) and *BANK1* (rs10516487G/A and rs3733197G/A) genotypes. The blue-green colors represent redundant. In our study, redundancy was observed due to high LD ($r^2 \approx 1$) between both *BLK* SNPs. Yellow shows independence. In addition, we did not observe a synergetic interaction between the four *BLK* and *BANK1* polymorphisms

Table 5 Haplotype frequencies and association analysis between the four *BLK* and *BANK1* SNPs in patients with SLE and controls

Haplotype	SLE (%)	Controls (%)	OR	95 % CI	*p*	*pc*
BLK						
TA	76.9	68.4	1.54	1.24–1.90	7.7×10^{-5}	3.0×10^{-5}
CG	22.5	31.6	0.62	0.50–0.77	2.0×10^{-5}	1.0×10^{-5}
BANK1						
GG	77.2	75.4	1.1	0.89–1.38	0.38	0.77
AA	7.9	13.0	0.57	0.41–0.79	0.0005	0.0015
GA	12.1	8.7	1.43	1.05–1.95	0.02	0.073
TG	2.9	2.9	1.02	0.58–1.78	0.96	1.0

The order to *BLK* is rs2736340T/C, and rs13277113A/G, meanwhile in *BANK1* is rs10516487G/A, and rs3733197G/A

OR odds ratio, *CI* confidence interval

*$p<0.05$, statistically significant, *pc* corrected *p* value after 100,000 permutations

between *BANK1* R61H and susceptibility to SLE under the allelic, codominant, dominant, and recessive models. The *BANK1* rs10516487A minor allele was associated with protection against SLE (data shown at the bottom of Table 3). Regarding haplotypes, we did not identify an association between the GG haplotype carrying the two major alleles and susceptibility to SLE; however, we found an association between the AA haplotype carrying the two *BANK1* rs10516487A and rs3733197A minor alleles and protection against SLE. Although we observed an association between the GA haplotype carrying the rs10516487G major allele and the rs3733197A minor allele and susceptibility to SLE, this was lost after correction by 100,000 permutations. This means that the association observed with susceptibility to SLE and *BANK1* is given by rs10516487A as we previously observed in the individual analysis for each SNP.

Our study is the first showing an association between *BANK1* R61H and *BLK* rs13277113A/G and rs2736340T/C and SLE in a Latin-American population. Two previous analysis carried out in Latin America did not identify an association of *BLK* rs13277113A/G and *BANK1* R61H and SLE (although one of them showed a trend towards an association in Argentina a population) [4, 30]. In that research, which included patients from Argentina, Peru and Mexico, no association was identified [30]. The difference between the study by Sánchez et al. and our study is the sample size in cases and controls obtained from Mexico (we included a greater number of cases and controls). In addition, our cases and controls are from central Mexico, while the previous study included a great majority of individuals (of a total of 373 patients with SLE and 272 controls) with Mexican ancestry who were born or living in the USA, as well as patients and controls from some regions of the Mexican Republic (101 patients with SLE and 64 controls) such as Guadalajara, Culiacan, Morelia and Mexico City [30]. Mexico is a country formed by a great admixed population and each state or region has a different ancestral component, e.g., in a study carried out in Mexico City (using autosomal ancestry-informative markers [AIMs]) was identified that in the population, ancestry was predominately Amerindian (50%), Caucasian (45%) and African (5%) [31]. Meanwhile, Guadalajara and Morelia (both located in the western Mexico), and Culiacan (located in the north of Mexico) have a high proportion of European ancestry [32, 33]. Finally, Sánchez et al. analyzed the data taking into account all the Hispanic patients and controls while we compared the genotype and allelic frequencies among individuals from central Mexico. Both Argentines and Peruvians have a different proportion of Caucasian, Amerindian or African ancestry versus Mexicans [31–35].

We also analyzed the interactions between the *BLK* and *BANK1* SNPs. The best model showed an epistatic interaction between the four *BLK* and *BANK1* SNPs. Additionally, our analysis showed an association between the genotypes: *BLK* rs2736340T/C-rs13277113A/G and *BANK1* rs10516487G/A-rs3733197G/A and susceptibility to SLE. Because *BLK* rs2736340T/C and rs13277113A/G were in high LD ($r^2\approx1$), we observed a redundant interaction; meanwhile, we did not identify a synergistic epistatic interaction between these four *BLK* and *BANK1* SNPs. Previously, other researchers observed an association between some interactions of *BLK–BANK1* and SLE as well as with RA [3, 14, 22, 36, 37]. Thus, our data are in accordance with these findings.

The BANK1 protein is primarily expressed in B cells and is involved in cell signaling and proliferation pathways [4]. Regarding the non-synonymous *BANK1* rs10516487G/A SNP (which leads to an amino acid change, the G allele encodes arginine, while the allele A encodes histidine), functional studies have shown that the *BANK1* rs10516487GG genotype produces more BANK1 mRNA and protein compared to the rs10516487AA genotype in cell lines [4, 38] while the *BANK1* rs10516487G allele has been associated with an increased potential for protein multimerization. In addition, this same variant affects the mRNA splicing [38].

Another study showed that this polymorphism affects the B-cell signaling and is associated with an increase in memory B cells [39].

On the other hand, although we identified a weak deviation from HWE in the distribution of genotypes of *BANK1* rs3733197G/A in controls ($p = 0.02$), we decided to evaluate this variant and found that it is not a risk factor to SLE (although a trend towards an association with susceptibility was observed). However, our result should be taken with caution due to the deviation from HWE that we observed. Previous studies found controversial results. A study of European ancestry reported an association between this SNP and SLE [4] while a study in a Chinese population did not replicate this finding [18]. Thus, our data are consistent with the Chinese study. Contradictory findings can be due to the sample size, statistical power, ancestry, HWE deviation, etc. Of note, the *BANK1* A383T polymorphism showed no association with SLE; however, we identified an association with susceptibility to SLE when this variant interacts with *BLK* rs13277113A/G-rs2736340T/C and *BANK1* rs10516487G/A. This finding is important because we ought to evaluate genetic interactions between variants that show or not a previous association with SLE (or with other multifactorial diseases) to determine a global risk for this AD.

Our study has some limitations. First, a few *BLK* and *BANK1* SNPs were evaluated; however, other polymorphisms in both genes have been associated with SLE and those variants were not included in this study [3, 4, 16, 40]. Second, we do not evaluate clinical characteristics (only LN) and we cannot exclude the role of these SNPs as modifiers of the disease. Thus, we cannot rule out possible associations between *BLK* or *BANK1* SNPs and various clinical and/or serological features of SLE. Third, the absence of AIMs could cause biases in our results, thus, our data should be taken with caution.

In summary, this is the first study showing an association of *BLK* rs2736340T/C and rs13277113A/G and *BANK1* R61H with susceptibility to SLE in a Latin-American population. In addition, some *BLK* and *BANK1* interactions were associated with susceptibility to SLE.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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