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ORIGINAL ARTICLE

TNFSF4 is a risk factor to systemic lupus erythematosus in a Latin American population

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Abstract

Objective The aim of this study was to examine the association of three *TNFSF4* single nucleotide variants (SNVs) with systemic lupus erythematosus (SLE) susceptibility in Mexican patients.

Methods Genotypes of the *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G SNVs were determined using a TaqMan assay. In our study, we included 395 patients with SLE and 500 controls.

Results Our information shows a significant difference in the allelic and genotypic frequency of the three *TNFSF4* SNVs between cases and controls. Thus, our data showed an association between *TNFSF4* rs1234315T/C (T vs. C, OR 1.40, $p = 0.00087$), rs2205960G/T (G vs. T, OR 1.32, $p = 0.0037$), and rs704840T/G (T vs. G, OR 1.41, $p = 0.0003$) and SLE susceptibility in Mexican subjects. Besides, we conducted a meta-analysis to determine the role of *TNFSF4* rs2205960G/T and SLE susceptibility; our results showed that this variant is a risk factor for SLE in Latin Americans and Asians.

Conclusion Our results show that *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G are risk factors to SLE in Mexicans. This is the first study to document an association between *TNFSF4* rs704840T/G and SLE in a Latin American population. In addition, our meta-analysis showed that *TNFSF4* rs2205960G/T is a risk factor for Asians and Latin Americans.

Key Point

• *The TNFSF4 rs1234315T/C, rs2205960G/T, and rs704849T/G SNVs are risk factors to SLE in patients from Mexico.*

Keywords Single nucleotide variants susceptibility · Systemic lupus erythematosus · Tumor necrosis factor ligand superfamily member 4

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Introduction

The systemic lupus erythematosus (SLE) represents the prototype of a systemic autoimmune disease (AD) characterized by impaired immune responses, autoantibody production, and multi-organ damage [1, 2]. This AD is influenced by both genetic and environmental factors [3]. Some studies have shown that the genetic factor confers high risk to develop SLE [3, 4]. Recently, some candidate gene and genome-wide association studies (GWAS) conducted in patients with SLE primarily from European- and Asian-derived populations have identified several single nucleotide variants (SNVs) located within *TNFSF4* confer susceptibility to this AD [5–8]; however, one the these GWAS conducted in patients with SLE from African- and Hispanic-derived populations did not replicate this finding [7]. In addition, another GWAS carried out in Latin Americans and the US patients also did not identify an association between this gene and SLE susceptibility [9].

On the other hand, two candidate gene studies carried out in Hispanic patients identified an association between *TNFSF4* rs2205960G/T and rs1234315T/C and SLE susceptibility. Although one of them included Mexican patients, the sample size is not mentioned in detail, and in the second study, the countries included were not indicated, so in both studies, the number of samples of Mexican patients is not well defined [10, 11].

TNFSF4 encodes the ligand for OX40 (OX40L), which is a member of the TNF superfamily that is expressed on antigen-presenting cells, such as dendritic cells [12], B cells [13], macrophages [14], mast cells [15], natural killer cells [16], and vascular endothelial cells [17]. Moreover, OX40 is more expressed on activated $CD4^+$ than $CD8^+$ T cells while in NK cells and neutrophils, it is lower [18]. OX40 is also a thymic T cell marker receiving positive selection signals [19]. OX40/OX40L engagement delivers a potent costimulatory signal to activated T effector cells, supporting their survival, differentiation, and transition to a memory phenotype [20]. Furthermore, OX40–OX40L interactions regulate the expansion, differentiation, and activity of T regulatory cells (Treg) in both positive and negative manners depending on the conditions or AD [21–23, 20].

Thus, because it is essential to define susceptibility genes for SLE in each ethnic group, here, we determine whether the *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G SNVs are risk factors to SLE in a Mexican population. Besides, we performed a meta-analysis to evaluate the association between *TNFSF4* rs2205960G/T and SLE risk because it is the most studied variant in European and Asian populations; however, in Latin Americans, it has been scarcely reported.

Material and methods

Study population

A total of 395 patients with SLE and 500 unrelated healthy individuals, all belonging to the Mexican population, were admitted from the Hospital Juárez de México. The diagnosis was made using the American College of Rheumatology (ACR) criteria for SLE [24]. Both cases and controls were Mexican–Mestizo women over 18 years of age from Central Mexico. All patients with SLE and controls were sex-matched. Unrelated healthy volunteers were also recruited from the same hospital; all healthy controls had no family history of ADs or inflammatory diseases such as asthma, obesity, type 2 diabetes, and chronic urticaria. Patients with SLE who presented another AD were excluded from our study. Both cases and controls had no infectious disorders. The research protocol has been previously reviewed and approved by the Biosecurity, Research, and Ethics Committees of the Hospital Juárez de Mexico (Protocol number: HJM 0446/18-I). All SLE patients and healthy controls included in our study signed an informed consent letter.

DNA sample extraction

Genomic DNA was extracted from whole blood samples using the standard phenol-chloroform technique. The purity and quantification of nuclear DNA were evaluated by spectrophotometry using a Nanodrop 2000 instrument (Thermo Fisher Scientific). DNA samples that had a 260/280-nm absorbance ratio between 1.8 and 2.0 and concentrations of ≥50 ng/μL were considered appropriate for the study. The extracted DNA samples were stored at − 20°C before genotyping.

Genotyping

Genotypes of the three *TNFSF4* SNVs were obtained with ready-to-order TaqMan assays from Thermo Fisher: rs2205960G/T (assay ID C___2955953_10), rs1234315T/C (assay ID C___8920846_10), and rs704840T/G (C___2469479_10). CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, CA, USA) was used according to the manufacturer's instructions to obtain *TNFSF4* fluorescence emission and the allelic discrimination plot. We always observed the three genotypes of each of the three *TNFSF4* SNVs in an allelic discrimination plot, which showed us that these variants are frequent in our population. Fifty percent of all the samples (including patients and controls) were genotyped twice (for the three polymorphisms); the reproducibility of the results was 100%.

Bioinformatics analysis

Regulatory and functional analysis of rs1234315, rs2205960, and rs704840 variants were done using RegulomeDB database [25] and HaploReg v4.1 tool [26], respectively. Correlations between SNPs and levels of TNFSF4 expression were identified using genotype-tissue expression (GTEx) data [27]. The expression of quantitative trait loci (eQTL) was analyzed to determine the functional role of phenotype-associated SNVs.

A meta-analysis of *TNFSF4* rs2205960G/T: identifying eligible studies, data extraction, evaluation of the statistical association, and publication bias

The keywords "*TNFSF4*" "rs2205960", "polymorphism" and "systemic lupus erythematosus" were searched in PubMed, Web of Science, and Google Scholar databases to identify different studies (up to June 2020). We had the following inclusion criteria: (a) case–control design; (b) data of genotypic and allelic frequencies, odds ratio (OR), 95% confidence interval (CI), and *p* value; and (c) the language was limited to English. Studies were excluded according to the following criteria: (a) no usable data reported by the study and (b) duplicate of previous publications. Two independent researchers collected all data. Our analysis included information of the first author, year of publication, ethnicity, country, and the number of genotypes in cases and controls . All statistical analysis was conducted using Metagenyo [28]. The heterogeneity effect was evaluated with the I^2 statistical (range was 0–100%). We used a random effects model when heterogeneity of $p < 0.1$ or $I^2 > 50\%$ was observed in our study; otherwise, the fixed effects model was used. This meta-analysis was compared to the genotypes and alleles of *TNFSF4* rs2205960 SNV. Thus, our analysis was conducted under the allelic, codominant, dominant, recessive, and overdominant models. We also evaluated the effect given by ethnicity (all cases and controls were divided into Asians and Latin Americans). Meanwhile, the sensibility analysis was used to determine the stability of our study. We did not include any studies of European populations because the published articles did not provide genotype information. However, the role of this variant in SLE susceptibility in these populations is well known.

Statistical analysis

In our quality-control filters, we did not remove variants in *TNFSF4*; however, we removed some samples because they did not amplify for one, two, or three *TNFSF4* variants (the genotyped success of the studied variants was higher than 97%). We eliminate *TNFSF4* variants with significant deviation from Hardy–Weinberg ($p < 0.05$) in controls. Besides, the allelic frequencies of the *TNFSF4* variants were compared with the reported from the 1000 Genome Project. The Chi-square test was used to compare the observed and expected genotype frequencies. Four models for genetic association analysis were used: the codominant, dominant (minor allele homozygotes plus heterozygotes vs. major allele homozygotes), recessive (minor allele homozygotes vs. heterozygotes plus major homozygotes), and allelic models. The Chi-square test compared the allelic and genotypic frequencies between cases and controls. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using the Finetti software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). This same software was used to evaluate the HWE and the genetic association under allelic and codominant models, meanwhile, Epidat V 3.1 (https://extranet.sergas.es/epiwb/EPIWB/DescargaEpidat.aspx?IdPaxina=62715&idv=1&lng=es) was used to evaluate the dominant and recessive genetic models. Bonferroni correction was performed in our study to determine the association of genotypes or alleles of the three *TNFSF4* SNVs with SLE. Linkage disequilibrium (LD) was measured using Haploview software (V 4.0); this same program was used to obtain the different *TNFSF4* haplotypes. A *p* value between 0.05 and 0.0166 (0.05/three SNVs) revealed a nominal association, mean; a *p* value < 0.0166 was considered to be statistically significant.

Result

Characteristics of cases and controls

A total of 395 cases with SLE and 500 healthy controls were included in our study. The characteristics of SLE and healthy controls are shown in Table 1. There were no significant differences between patients and controls in terms of sex or mean age distributions (all $p > 0.05$). Our study group was of Latin ancestry and representative of a Mexican population. The SNVs investigated in this study fulfilled HWE in the control group (*p* values > 0.05).

Table 1 Demographic characteristics in patients with SLE and controls

	Controls *n* = 500 (%)	SLE *n* = 395 (%)
Age (mean ± SD years)	52.7 ± 7.9	38 ± 12
Gender (Female)	100	100

SLE systemic lupus erythematosus, SD standard deviation.

Table 2 Genotypic and allelic frequencies of the *TNFSF4* polymorphisms and association analysis in patients with SLE and healthy individuals

TNFSF4 SNV	Model	Genotype or allelle	SLE *n* (%)	Controls *n* (%)	OR 95% CI	*p*
rs1234315T/C	Codominant	TT	191 (49.6)	190 (38.1)	1.69 (1.13–2.53)	0.01
		TC	144 (37.4)	224 (45.0)	1.08 (0.72–1.62)	NS
		CC	50 (13.0)	84 (16.9)	–	
	Allelic	T	526 (68.3)	604 (60.6)	1.40 (1.15–1.70)	0.00087
		C	244 (31.6)	392 (39.4)	–	
	Dominant	CC	50(13.0)	84 (16.9)		
		TC + TT	335 (87.0)	414 (83.1)	1.36 (0.93–1.98)	NS
	Recessive	TC + CC	194 (50.4)	308 (61.8)		
		TT	191 (49.6)	190 (38.2)	1.60 (1.22–2.09)	0.0007
rs2205960G/T	Codominant	GG	120 (30.4)	187 (37.6)	–	–
		GT	186 (47.1)	230 (46.3)	1.26 (0.93–1.70)	NS
		TT	89 (22.5)	80 (16.1)	1.73 (1.19–2.53)	0.0043
	Allelic	G	426 (53.9)	604 (60.8)	–	–
		T	364 (46.1)	390 (39.2)	1.32 (1.09–1.60)	0.0037
	Dominant	GG	120 (30.4)	187 (37.6)	–	
		GT + TT	275 (69.6)	310 (62.4)	1.38 (1.04–1.83)	0.0236
	Recessive	GG + GT	306 (77.5)	417 (84.0)	–	
		TT	89 (22.5)	80 (16.0)	1.52 (1.08–2.12)	0.015
rs704840 T/G	Codominant	TT	88 (22.4)	148 (29.6)	–	
		TG	179 (45.4)	240 (48.0)	1.25 (0.90–1.74)	NS
		GG	127 (32.2)	112 (22.4)	1.91 (1.32–2.75)	0.0005
	Allelic	T	355 (45.0)	536 (53.6)	–	
		G	433 (55.0)	464 (46.4)	1.41 (1.17–1.70)	0.0003
	Dominant	TT	88 (22.3)	148 (29.6)	–	
		TG + GG	306 (77.7)	352 (70.4)	1.46 (1.08–1.98)	0.014
	Recessive	TT + TG	267 (67.8)	388 (77.6)		
		GG	127 (32.2)	112 (22.4)	1.65 (1.22–2.22)	0.001

Allele, genotype, and haplotypes frequencies and analysis association between cases and controls

The allele and genotype distributions of the three *TNFSF4* SNVs are presented in Table 2. The *TNFSF4* rs1234315C/C or C minor genotype or allele, respectively, was used as a reference to determine the odds of acquiring SLE. We observed a higher distribution of *TNFSF4* rs1234315T allele in patients with SLE than controls (OR 1.40, 95% CI 1.15–1.70, $p = 0.00087$, Table 2). Additionally, there were also differences between patients and controls in the genotype frequency distributions between cases and controls (Table 2). We also identified an association with SLE under the recessive model ($p = 0.0007$, Table 2). These findings show that the *TNFSF4* rs1234315T allele increases the susceptibility of SLE in our population. Besides, we identified a frequency of rs1234315T allele of 60.6% in our controls, similar information (57.8%) was reported in individuals with Mexican ancestry living in Los Angeles (data from the 1000 Genome Project to Mexicans).

On the other hand, the *TNFSF4* rs2205960G/T and rs704840T/G variants were successfully genotyped in virtually all cases and controls. The allele and genotype distributions of both *TNFSF4* SNVs are presented in Table 2. The major genotype or allele of both *TNFSF4* SNVs were used as a reference to determine the odds of acquiring SLE. The frequencies of the three genotypes of *TNFSF4* rs2205960G/T in patients with SLE were as follows: GG 120 (30.4%), GT 186 (47.1%), and TT 89 (22.5%), respectively, whereas in healthy controls, the frequencies were GG 187 (37.6%), GT 230 (46.3%), and TT 80 (16.1%), respectively. Thus, the *TNFSF4* rs2205960TT genotype showed a significant association with susceptibility to SLE; OR 1.73, 95% CI 1.19–2.53, $p = 0.0043$ (Table 2). Additionally, the rs2205960T allele also showed a significant difference between cases and controls, 364 (46.1%) and 390 (39.2%), respectively, and an

association with SLE was observed (Table 2). We also observed an association with susceptibility to SLE under the recessive model and a nominal association under the dominant model (Table 2). We also identified a similar frequency of the *TNFSF4* rs2205960T allele (33.6%) in the data from the 1000 Genome Project to Mexicans (in our controls, we identified 39.2%).

On the other hand, the frequencies of the three genotypes of the *TNFSF4* rs704840T/G SNV in SLE were as follows: TT 88 (22.4%), TG 179 (45.4%), and GG 127 (32.2%), respectively, whereas in healthy controls, the frequencies were TT 148 (29.6%), TG 240 (48.0%), and GG 112 (22.4%), respectively. The *TNFSF4* rs704840G allele also showed a significant difference in both cases and controls (Table 2). Thus, either *TNFSF4* rs704840GG or G showed an association with susceptibility to SLE; TT vs. GG: OR = 1.91, 95% CI 1.32–2.75, $p = 0.0005$, and T vs. G; OR 1.41, 95% CI 1.17–1.70, $p = 0.0003$ (Table 2). We also observed an association under the dominant and recessive models (Table 2). When comparing the frequency of *TNFSF4* rs704840G identified in our controls (46.6%) with the reported in the 1000 Genome Project in Mexicans (39.1%), although apparently, it seemed to differ; we analyze these frequencies, and we did not identify a statistically significant difference (data not shown).

Regarding haplotypes, we identified seven different allele combinations (Table 3), but only one of them showed association with susceptibility to SLE; the TTG haplotype, which carries the three risk alleles (rs1234315T/C, rs2205960G/T, and rs704840T/G). However, this association was lost after applying a correction with 100,000 permutations. Our data show that these *TNFSF4* SNVs are not in LD (Fig. 1), indicating that the association observed between these *TNFSF4* SNVs and SLE susceptibility is independent of each genetic marker.

Table 3 Haplotype frequencies and association analysis between TNFSF4 haplotypes in women with SLE and in controls

Haplotype	SLE (%)	Controls (%)	OR	95% CI	*p*	*pc*
TTG	42.9	36.9	1.29	(1.06–1.54)	0.01	NS
CGT	28.2	36.4	0.69	(0.56–0.84)	0.0003	0.0009
TGT	15.1	15.9	0.94	(0.72–1.21)	NS	NS
TGG	8.9	7.0	1.29	(0.91–1.82)	NS	NS
CGG	1.8	1.6	1.10	(0.54–2.30)	NS	NS
CTG	1.4	0.9	1.55	(0.64–3.70)	NS	NS
TTT	1.4	0.8	1.75	(0.70–4.40)	NS	NS

The order is as follows: rs1234315T/C, rs2205960G/T, and rs704840T/G.

CI confidence interval, *OR* odds ratio, *pc* corrected *p* value after 100,000 permutations

eQTL effect of *TNFSF4* variants

Functional analysis of rs2205960, rs1234315, and rs704840 variants using HaploReg V4.1 [26] tool shows enhancer histone marks, DNAse, and/or motifs changed, suggesting that the three SNPs could affect the expression of TNFSF4 by influencing its promoter activity (Supplementary table S1). However, further functional experiments are needed to confirm their functional status. RegulomeDB [25] was used to annotate genetic variants studied with known and predicted regulatory elements. The results showed that the three genetic variants showed a DNase hypersensitivity, and they were

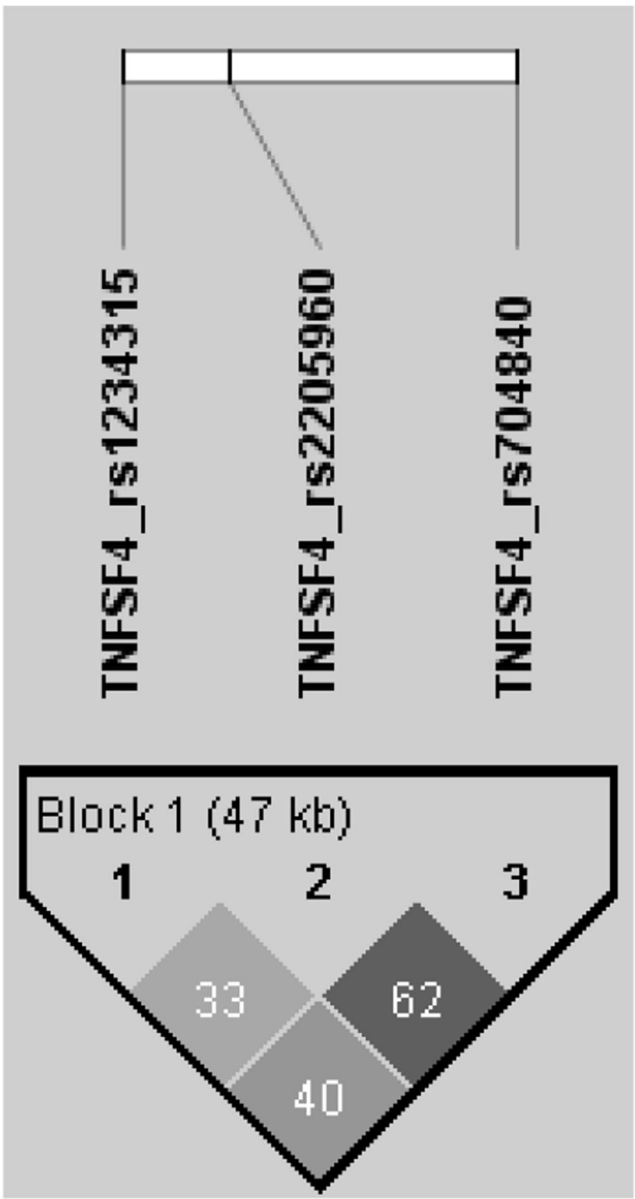

Fig. 1 LD between the three *TNFSF4* SNVs in cases with SLE and controls. Our data showed no LD between these genetic markers ($r^2 \leq 80\%$); thus, the associations identified between these *TNFSF4* SNVs and SLE were independent because these did not segregate together

located in transcription factor binding sites (Supplementary table S1). To consider the potential influence of genetic variation on gene expression, we performed an eQTL analysis to determine whether these genetic variants would alter TNFSF4 mRNA expression in different tissues. However, we have not found ($p > 0.05$) that the SNVs rs1234315, rs2205960, and rs704840 altered TNFSF4 expression in different tissues using GTEx [27] (Supplementary figure S1).

Meta-analysis of *TNFSF4* rs2205960T/G in SLE

We identified a total of 21 citations after an initial search of articles related to *TNFSF4* rs2205960T/G and SLE susceptibility. However, only four showed information on the number of genotypes and alleles: one Latin American [11] and three Asians [29–31]; thus, these studies (and our current study) were finally included in the meta-analysis [29–31, 11]. Data from included studies as well as HWE are shown in Table 4. In our meta-analysis, included were 16,427 unrelated individuals, 6,129 patients with SLE, and 10,298 controls. We identified heterogeneity in allelic contrast of $I^2 = 25\%$ and $p = 0.238$ (Fig. 2). We also observed similar data for the recessive, dominant, codominant, and overdominant models (Table 5). Thus, our meta-analysis was conducted using a fixed effects model (Table 5). The sensitive analysis was applied to detect the influence of each case–control study included in the meta-analysis; therefore, each of the studies should be omitted at a time, mean the pooled OR, and its 95% CI would be recalculated again. Our results showed an association of *TNFSF4* rs2205960G/T in Asians and Latin Americans (Supplementary figure S2).

Publication bias was applied to our analysis by generating a funnel plot for this variant under the different genetic models; after analysis, we did not identify any publication bias for the association of *TNFSF4* rs2205960G/T and SLE under the allele model (Fig. 3) (Egger's test; p value = 0.6464); similar data were identified for the recessive, dominant, codominant, and overdominant models (Supplementary figure S3). Finally, in the ethnicity analysis, we observed an association in the overall and stratified (in Asians and Latin Americans) analyses (Table 5).

Discussion

Epidemiological studies of SLE have led to an increased interest in studying the genetic basis of this AD. Genetic association studies have identified novel SLE susceptibility genes, including *TNFSF4*, which encodes for OX40L protein that causes the imbalance of T cell activation and can increase autoantibody production [20].

Several *TNFSF4* SNVs have been identified through GWA and candidate gene studies to be associated with SLE severity or susceptibility, primarily in Asian- and European-derived communities [6, 32, 33, 5, 34]. Similar to these studies, we also identify an association between the *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G SNVs and susceptibility to SLE. Thus, in the Mexican population, all these variants evaluated in our study confer risk to develop this AD.

Table 4 Studies included in our meta-analysis

Author	Year	Ethnicity	Source	Genotyping method	GG cases	GT cases	TT cases	GG controls	GT controls	TT controls	HW p value	HW-adjusted p value
Sanchez et al.	2010	Latin American	HB	Illumina Custom Bead	310	381	103	329	265	55	0.874	0.9855
Zuo et al.	2014	Asian	HB	Illumina Human610-Quad BeadChips and Sequenom MASSArray	1751	1815	504	4311	3174	575	0.7796	0.9855
Chua et al. 1	2016	Asian	HB	TaqMan	171	132	33	203	134	22	0.9855	0.9855
Chua et al. 2	2016	Asian	HB	TaqMan	71	34	4	76	33	5	0.5629	0.9851
Chua et al. 3	2016	Asian	HB	TaqMan	12	14	5	24	12	0	0.2301	0.9851
Gupta et al.	2018	Asian	HB	TaqMan	213	144	37	353	198	32	0.5421	0.9851
Present study	2020	Latin American	HB	TaqMan	120	186	89	187	230	80	0.5113	0.9851

HB hospital based

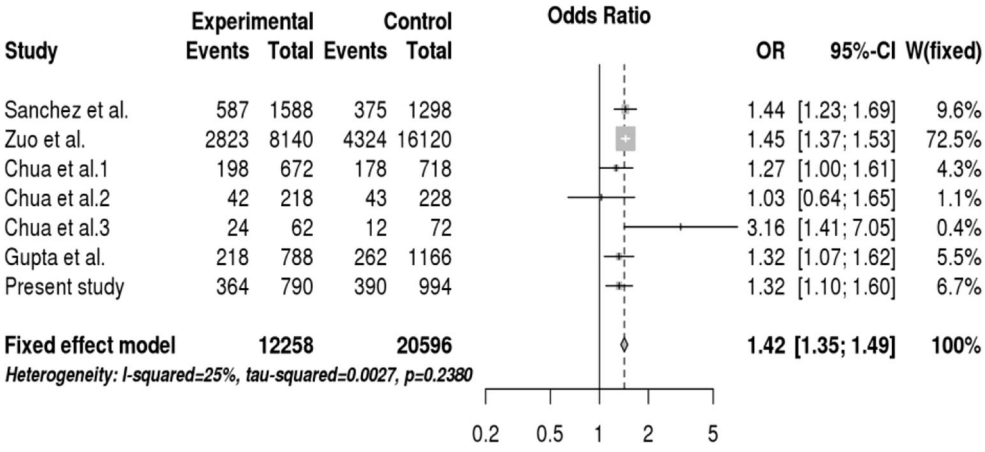

Fig. 2 Forest plot of *TNSFS4* rs2205960G/T associated with SLE in samples from Asians and Latin Americans

It has been shown through *in silico* analysis that the *TNFSF4* rs2205960T allele has a higher binding affinity to NF-kB p65 protein compared to G allele, which suggests that the *TNFSF4* rs2205960T allele possesses a regulatory effect in gene expression; however, functional studies are ought to be carried out to determine the biological role of this variant [10]. Regarding the other two variants, rs1234315T/C and rs704840T/G, as far as we know, there are no reported studies about its functional relevance. We conducted *in silico* regulatory (eQTLs) and functional analyses of three *TNFSF4* SNVs studied using HaploReg V4.1 [26] and RegulomeDB V2.0 [25], which suggest they are functional variants in SLE patients. However, eQTL analysis showed no evidence on TNFSF4 expression; therefore, further study is necessary to confirm the function of these variants in lupus. Thus, other variants (of *TNFSF4* or another nearby gene) different from

Table 5 Summary of the subgroup analyses of *TNFSF4* rs2205960G/T in our meta-analysis

rs2205960	Population	No. of studies	Model	Test of association			Heterogeneity		Publication bias
				OR	95% CI	*p* value	I^2	*p* value	Egger's test *p* value
Allelic model (T vs. G)	Overall	7	F	1.42	1.35–1.49	<0.0001	0.25	0.238	0.64
	Asian	5	F	1.42	1.35–1.51	<0.0001	0.46	0.117	0.79
	Latin American	2	F	1.39	1.23–1.57	<0.0001	0	0.489	NA
Recessive model (TT vs. TG + GG)	Overall	7	F	1.77	1.59–1.97	<0.0001	0	0.57	0.709
	Asian	5	F	1.82	1.62–2.05	<0.0001	0	0.49	0.997
	Latin American	2	F	1.56	1.23–1.57	<0.0003	0	0.81	NA
Dominant model (TT + TG vs. GG)	Overall	7	F	1.49	1.40–1.59	<0.0001	0.13	0.33	0.569
	Asian	5	F	1.49	1.39–1.59	<0.0001	0.34	0.19	0.617
	Latin American	2	F	1.52	1.29–1.80	<0.0001	0	0.40	NA
Overdominant model (TG vs. TT + GG)	Overall	7	F	1.22	1.14–1.30	<0.0001	0	0.70	0.48
	Asian	5	F	1.22	1.14–1.31	<0.0001	0	0.82	0.518
	Latin American	3	F	1.21	1.03–1.43	0.023	0.55	0.13	NA
TT vs. GG	Overall	7	F	2.07	1.85–2.31	<0.0001	0	0.46	0.598
	Asian	5	F	2.12	1.87–2.39	<0.0001	0.14	0.33	0.835
	Latin American	2	F	1.86	1.43–2.42	<0.0001	0	0.61	NA
TT vs. TG	Overall	7	F	1.49	1.33–1.67	<0.0001	0	0.78	0.897
	Asian	5	F	1.53	1.35–1.74	<0.0001	0	0.67	0.81
	Latin American	2	F	1.34	1.04–1.73	<0.025	0	0.83	NA
TG vs. GG	Overall	7	F	1.38	1.29–1.48	<0.0001	0	0.55	0.516
	Asian	5	F	1.38	1.28–1.48	<0.0001	0	0.43	0.523
	Latin American	2	F	1.43	1.19–1.70	<0.0001	0.014	0.32	NA

F fixed effect model

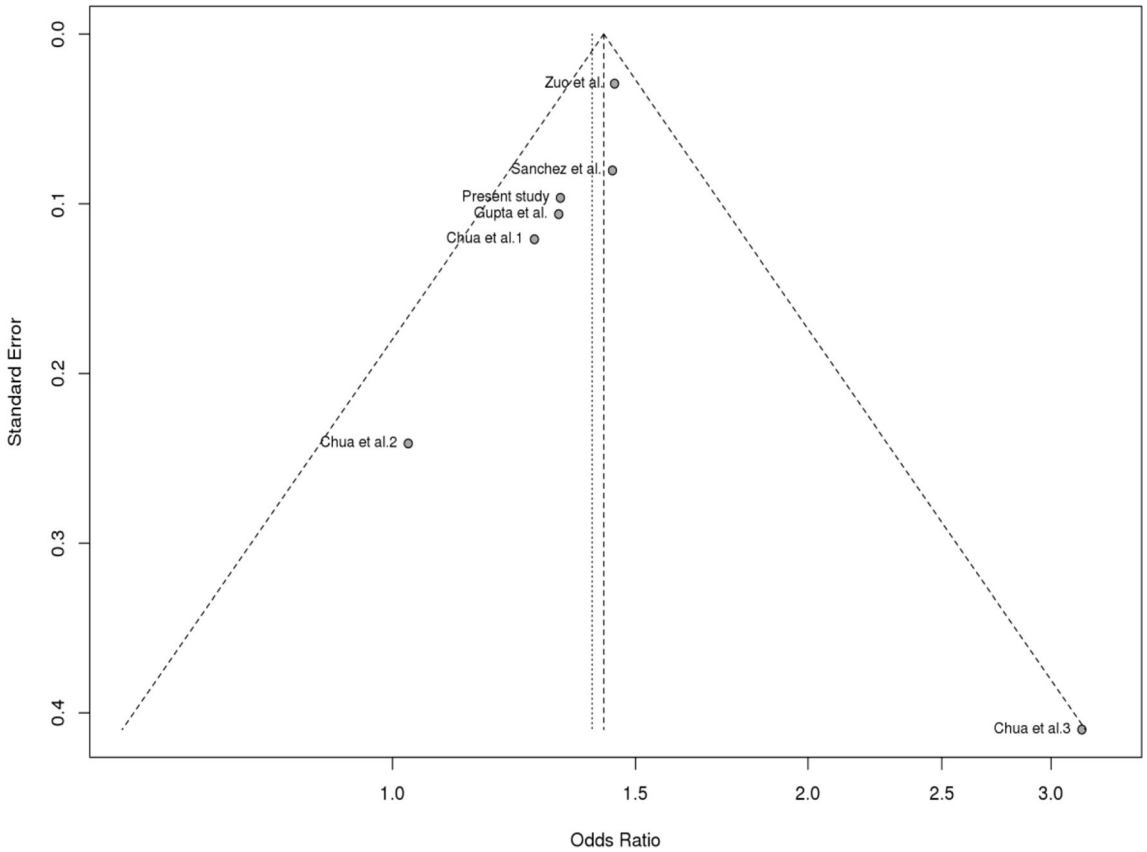

Fig. 3 Funnel plots under the allelic genetic model between *TNFSF4* rs2205960G/T and SLE

those we have analyzed in our current study may be the direct causes of SLE's susceptibility.

Our results showed that the *TNFSF4* rs2205960T minor allele was distributed more frequently in patients with SLE compared to healthy controls in the Mexican population. A similar condition was previously reported in patients from the UK and Minnesota [5], Germany, Italy, and Argentina (in this last study, it was evaluated that the *TNFSF4* rs12039904 [genotyped] SNV, which is in high LD with rs2205960 [imputed]) [35], from the USA, Sweden, East Asian, Hispanics, or other Caucasian groups [10, 33, 36], African Americans [37], as well as in different Asian populations including India and China [29]. Besides, two candidate gene studies evaluated the *TNFSF4* rs2205960G/T variant and replicated this finding in Hispanic patients with SLE; one of them included Mexican patients; however, the number of samples is not described in detail. Additionally, 101 SLE patients from different regions from Mexico (patients from Guadalajara, Morelia, Culiacán, and Mexico City) were included in this same study; meanwhile, the second study does not mention details about the Latin American countries included [10, 11]. On the other hand, two GWAS (both studies included samples from Mexico) conducted in Hispanic patients showed no association between *TNFSF4* SNVs and SLE [9, 7]; in both studies, the exact number of Mexican patients is not clear. These contradictory data in the GWA or candidate gene studies maybe because (1) those variants were removed from GWAS because of their high error rates; (2) there is an excess missing genotype; or (3) because they had no associations at a genome-wide significance level, among others. Due to these results, we here determine the role of the *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G SNVs on SLE susceptibility in Mexican patients. Our data also show that these three *TNFSF4* SNVs are risk factors for SLE, a result similar to that previously identified by Sánchez et al. [11] in Hispanic patients where Mexican individuals were included.

On the other hand, the *TNFSF4* rs1234315T/C SNV has also been associated with SLE in Hispanics, African Americans, Europeans, and Asians [10], meanwhile, *TNFSF4* rs704840T/G has shown an association with SLE in several Asian populations [6, 38], except in patients from India, Malaysia, and China [29]. Thus, our data is similar to these previously published. That is, both *TNFSF4* rs1234315T/C and rs704840T/G variants are risk factors to SLE. The functional effect of both variants as far as we know remains unknown; however, an *in silico* analysis suggests that rs1234315T/C can affect various biological aspects of this gene [39].

Because rs2205960 has been extensively evaluated and associated in most studies that include Caucasian and Asian patients with SLE, but not in Latin Americans, due to the lack of information in these populations, we performed a meta-analysis including our patients, under our inclusion criteria. The overall and stratified meta-analysis showed that *TNFSF4* rs2205960 is associated with SLE risk; thus, we identified that *TNFSF4* rs2205960 is a risk factor for Asians and Latin Americans.

The importance of replication in a much different population, in terms of validation of an association and discovery of population differences, should not be overlooked [40, 41]. Population differences in susceptibility genes may enlighten some genetic risks that are specific toward certain ethnic groups, which may also help elucidate the ethnic differences in terms of disease prevalence and susceptibility.

Although we identify an association between these three *TNFSF4* SNVs and SLE susceptibility, it is important to mention that our study presents some limitations, which may bias our results, for example the absence of ancestry informative markers and clinical data to perform the genotype–phenotype correlation. However, previous associations of this gene (in addition to the association that we identify in our study population) in Hispanic patients confirm that *TNFSF4* is an important SLE susceptibility locus in virtually all populations studied.

Conclusion

Our data show that the *TNFSF4* rs1234315T/C, rs2205960G/T, and rs704840T/G SNVs are risk factors to SLE in patients from Mexico. In addition, our meta-analysis shows that *TNFSF4* rs2205960T/G is a risk factor to SLE in Asians and Latin Americans. Our study is also the first to document an association between *TNFSF4* rs704840T/G and SLE susceptibility in a Latin American population.

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Compliance with ethical standards

Disclosures None

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