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ORIGINAL ARTICLE

TLR4 and *TLR9* polymorphisms are not associated with either rheumatoid arthritis or systemic lupus erythematosus in Mexican patients

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Abstract

Toll-like receptor (TLR)-mediated signaling pathways induce a proinflammatory microenvironment to eradicate pathogens. However, in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), TLRs can promote chronic inflammation. It has been shown that some *TLR4* and *TLR9* single nucleotide polymorphisms (SNPs) are risk factors for RA and SLE, but these findings have not been replicated in all populations; thus, results are inconclusive. We evaluated the *TLR4* Asp299Gly, Thr399Ile, – 1892G/A SNPs, and the *TLR9* Pro545Pro SNP to assess potential associations with RA and SLE in Mexican patients. This study included 474 patients with RA, 283 patients with SLE, and 424 healthy controls. We used a 5′ nuclease allelic discrimination assay to genotype individuals for the four *TLR4* and *TLR9* polymorphisms. We found that the genotype or allelic frequencies of the *TLR4* Asp299Gly, Thr399Ile, – 1892G/A, and *TLR9* Pro545Pro polymorphisms were similar between patients and controls. We found no association under different genetic models. A haplotype analysis of *TLR4* showed no association with either RA or SLE. We found no significant differences in the allelic or genotypic frequencies of *TLR4* Asp299Gly, Thr399IIe, – 1892G/A, or *TLR9* Pro545Pro between patients and controls. These findings suggested that these variants are not risk factors for RA or SLE in Mexican patients.

Keywords *TLR4* · *TLR9* · Single nucleotide variants · Rheumatoid arthritis · Systemic lupus erythematosus · Susceptibility

Introduction

Toll-like receptors (TLRs) are fundamental in the innate immune response, because they induce phagocytosis, the production of proinflammatory cytokines, etc. [1]. In addition, it has been shown that TLRs lead to the generation of apoptotic bodies, hypomethylated CpG islands, etc., which induce TLR-mediated inflammatory signaling pathways. TLRs and TLR-ligands also participate in breaking the tolerance to self-antigens and promoting inflammatory responses [1–3]. For example, it has been reported that TLR4 expression is increased in various immune cells of patients with different autoimmune diseases (ADs) [1]. In addition, it has also been shown that different single nucleotide polymorphisms (SNPs) affected *TLR4* function, including rs4986790 (Asp299Gly) and rs4986791 (Thr399Ile). These SNPs have been associated with both rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in a few populations [4–6]. However, because *TLR4* polymorphisms have rarely been evaluated in both RA and SLE, the data are inconclusive. Moreover, different studies have shown associations between *TLR4* Asp299Gly and reductions in the risk of RA [4]; the susceptibility to RA [7], and the severity of RA [6], but other studies could not replicate the findings [8–12]. Additionally, although the *TLR4* Thr399IIe SNP has been evaluated in RA, it has not shown an association with this AD [6–8, 10, 11], mean, the – 1892G/A (rs10983755) polymorphism has not been evaluated in this AD yet.

A few studies have evaluated the *TLR4* Asp299Gly and/or Thr399Ile SNPs in patients with SLE; however, the results were inconclusive. Only one study, carried out in an Indian

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population, showed an association between SLE and *TLR4* Asp299Gly [5, 8, 13]. The other two studies, carried out in Caucasians and Africans, failed to replicate that association [8, 13]. To the best of our knowledge, *TLR4* – 1892G/A has not been evaluated in patients with SLE to date.

Only one study investigated *TLR9* Pro545Pro in RA, and it did not report an association [12]. However, *TLR9* Pro545Pro conferred the risk of SLE in Chinese [14–16] and African [17] populations, but not in Brazilian, Polish, or Korean [18–20] populations. *TLR4* and *TLR9* polymorphisms have rarely been investigated in both RA and SLE. Therefore, here, we evaluated four polymorphisms to determine their potential roles in RA and SLE susceptibility in a Latin American population.

Materials and methods

We enrolled 474 patients with RA, 283 patients with SLE, and 424 controls with no history of ADs or chronic inflammation. Patients with RA and SLE were selected and recruited according to the ACR-EULAR 2010 and SLICC 2012 criteria, respectively. Patients with both RA and SLE were excluded from the study. Our original study population had a 9.4:0.6 female to male ratio; therefore, we decided to include only females both in cases and controls to avoid biasing the results. All patients and controls were over 18 years old and unrelated to each other. All participants provided informed consent.

We performed the following protocols as previously described [21]: DNA isolation, PCR reactions, *TLR4* polymorphism genotyping (rs4986790A/G: Asp299Gly; rs4986791C/T: Thr399Ile; and rs10983755G/A: – 1892), *TLR9* polymorphism genotyping (rs352140G/A: Pro-545Pro), and the TaqMan allelic discrimination assays.

Hardy–Weinberg equilibrium (HWE) values for each of the SNPs in the control group were obtained with Finetti software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). A p-value < 0.05 indicated a deviation from HWE. We calculated odds ratios (ORs) and p-values with Epidat software (V 3.1) (http://www.sergas.es/MostrarContidos_N3_T01.aspx?IdPaxina=62715). The four *TRL4* and *TLR9* SNPs were evaluated with allelic, codominant, dominant, and recessive models. Haplotype analyses and linkage disequilibrium (LD) calculations were performed with Haploview (V4.2) software, as previously described [21].

Results

Clinical and demographic data of patients with RA and SLE are shown in Table 1. The genotypic distributions of the *TLR4* and *TLR9* polymorphisms were in HWE in both patient and control groups. The genotypic and allelic frequencies of *TLR4* rs4986790A/G, rs4986791C/T, rs10983755G/A, and *TLR9* rs352140G/A were distributed similarly in patients with RA, SLE, and controls (Tables 2 and 3, respectively). Thus, we could not identify an association between the SNPs and either of the ADs, under the different genetic models. To note, no patient or control individual was homozygous for any of the minor genotypes; *TLR4* rs4986790A/G, rs4986791C/T, or rs10983755G/A; consequently, we could not test the recessive model (Tables 2 and 3). We also analyzed whether *TLR4* or *TLR9* variants were associated with serological markers for RA and SLE or with lupus nephritis. However, we did not identify any association (data not shown). Moreover, we did not observe any association between *TLR4* haplotypes and RA or SLE. We did not detect LD among the three *TLR4* SNPs (data not shown).

Table 1 Demographic characteristics and serological markers in RA and SLE patients

RA patients (n =)	474	283
Age (SD)	54.4 ± 12.8	38 ± 12.6
Sex (women); n (%)	474 (100%)	283 (100%)
Age at diagnosis (years)	39.8 ± 12.8	31.3 ± 12
RF^+ n (%)	362 (76.58%)	–
Anti-Ana	–	236 (83.39%)
LN	–	125 (44.16%)

RA rheumatoid arthritis, *SLE* systemic lupus erythematosus, *RF* rheumatoid factor, *LN* lupus nephritis

Discussion

TLR-mediated signaling pathways are activated by multiple foreign agents. This activation results in phagocytosis, cytokine secretion, lymphocyte activation, and chronic inflammation in RA and SLE [1–3]. Genetic alterations can affect the normal functions of different TLRs, including *TLR4* [22–26]. For example, studies have shown that *TLR4* Asp299Gly and Thr399Ile variants caused hyporesponsiveness to lipopolysaccharides [22] and impaired TLR4/MD-2 responses by altering ligand-dependent dimerization [23]. However, other studies have shown that Asp299Gly, but not Thr399Ile, could affect the binding of ligands and TLR4-mediated signaling [25]. Moreover, the *TLR4* – 1892G/A SNP appeared to have no effect on gene expression [24].

Three previous studies showed that *TLR4* Asp299Gly was associated with protection against, susceptibility to, and severity of RA in individuals from the Netherlands, China, and Poland, respectively [4, 6, 7]. However, those findings were not replicated in other populations, including another study conducted in the Netherlands [8–12]. In

Table 2 Genotypic and allelic frequencies of the *TLR4* and *TLR9* SNVs and association analysis in patients with RA and controls

Gene SNV	Model	Genotypes or alleles	RA n(%)	Controls n (%)	OR 95% CI	*P*
TLR4	Co-dominant	AA	452 (95.4)	405 (95.5)	Reference	–
rs4986790		AG	22 (4.6)	19 (4.5)	1.04 (0.55–1.95)	0.91
A/G		GG	0 (0)	0 (0)	–	–
	Allelic	A	926 (97.7)	829 (97.8)	Reference	–
		G	22 (2.3)	19 (2.2)	1.04 (0.56–1.93)	0.91
	Dominant	AA	452 (95.4)	405 (95.5)	Reference	–
		AG + GG	22 (4.6)	19 (4.5)	1.04 (0.55–1.95)	0.91
	Co-dominant	CC	456 (96.2)	407 (96.0)	Reference	
rs4986791		CT	18 (3.8)	17 (4.0)	0.95 (0.48–1.86)	0.87
C/T		TT	0 (0)	0 (0)	–	–
	Allelic	C	930 (98.1)	831 (98.0)	Reference	–
		T	18 (1.9)	17 (2.0)	0.95 (0.48–1.85)	0.87
	Dominant	CC	456 (96.2)	407 (96.0)	Reference	–
		CT + TT	18 (3.8)	17 (4.0)	0.95 (0.48–1.86)	0.87
	Co-dominant	GG	464 (97.9)	410 (96.7)	Reference	–
rs10983755		GA	10 (2.1)	14 (3.3)	0.63 (0.28–1.44)	0.27
G/A		AA	0 (0)	0 (0)	–	–
	Allelic	G	938 (98.9)	834 (98.3)	Reference	–
		A	10 (1.1)	14 (1.7)	0.64 (0.28–1.44)	0.27
	Dominant	GG	464 (97.9)	410 (96.7)	Reference	–
		GA + AA	10 (2.1)	14 (3.3)	0.63 (0.28–1.44)	0.27
TLR9	Co-dominant	TT	130 (27.4)	125 (29.5)	Reference	–
rs352140		TC	239 (50.4)	204 (48.1)	1.13 (0.83–1.53)	0.45
C/T		CC	105 (22.2)	95 (22.4)	1.06 (0.73–1.54)	0.75
	Allelic	T	499 (52.6)	454 (53.5)	Reference	–
		C	449 (47.3)	394 (46.5)	1.04 (0.86–1.25)	0.70
	Dominant	TT	130 (27.4)	125 (29.5)	Reference	–
		TC + CC	344 (72.6)	299 (70.5)	1.11 (0.83–1.48)	0.50
	Recessive	TT + TC	369 (77.8)	329 (77.6)	Reference	–
		CC	105 (22.2)	95 (22.4)	0.98 (0.72–1.35)	0.93

SNVs Single nucleotide variants, *OR* odds ratio, *CI* Confidence interval, *RA* rheumatoid arthritis

contrast, to date, no study has shown an association between *TLR4* Thr399IIe and RA [8, 10, 11]. Our results were consistent with previous findings that showed no association between *TLR4* variants and RA. Although *TLR4* – 1892G/A was shown to be a risk factor for chronic inflammation in pathogen-induced diseases [26], no study has reported its role in RA (or SLE) susceptibility. Additionally, the *TLR9* Pro545Pro variant has only been evaluated in one study that investigated patients with RA, and no association was identified [12]. Similarly, the present study could not identify any association between RA and the *TLR4* – 1892G/A or *TLR9* Pro545Pro variant. Our finding for *TLR4* Pro545Pro was similar to that reported in a French population [12].

Only one study conducted in an Indian population identified an association between SLE and *TLR4* Asp299Gly [5]; however, that finding was not replicated in Spanish or Tunisian populations [8, 13]. Similarly, *TLR4* Thr399IIe was only evaluated in two studies on SLE, and no association was reported [5, 8]. The present study investigated Mexican patients with SLE, and our results agreed with both previous studies. We found no association between SLE and either *TLR4* Asp299Gly or Thr399IIe. On the other hand, although *TLR4* – 1892G/A was previously associated with chronic inflammation [26], to our knowledge, it has not been evaluated in patients with SLE. In the present study, our data indicated that *TLR4* – 1892G/A was not a risk factor for SLE.

Previously, *TLR9* Pro545Pro showed associations with SLE in three different subgroups of a Chinese population and in Tunisians [14–17]. However, no association was found in Brazilian, Polish, or Korean populations [18–20]. Similarly, we did not identify an association in our population. However, the Tunisian and Chinese populations, where *TLR9* Pro545Pro was associated with SLE, had different ancestries than the ancestry of our population.

Table 3 Genotypic and allelic frequencies of the *TLR4* and *TLR9* SNVs and association analysis in patients with SLE and controls

Gene SNV	Model	Genotypes or alleles	SLE n(%)	Controls n (%)	OR 95% CI	*p*
TLR4	Co-dominant	AA	268 (94.7)	405 (95.5)	Reference	–
rs4986790		AG	15 (5.3)	19 (4.5)	1.19 (0.60–2.39)	0.62
A/G		GG	0 (0)	0 (0)	–	–
	Allelic	A	551 (97.3)	829 (97.8)	Reference	–
		G	15 (2.7)	19 (2.2)	1.19 (0.60–2.36]	0.62
	Dominant	AA	268 (94.7)	405 (95.5)		
		AG + GG	15 (5.3)	19 (4.5)	1.19 (0.60–2.39)	0.62
	Co-dominant	CC	271 (95.8)	407 (96.0)	Reference	–
rs4986791		CT	12 (4.2)	17 (4.0)	1.06 (0.50–2.25)	0.88
C/T		TT	0 (0)	0 (0)	–	–
	Allelic	C	554 (97.9)	831 (98.0)	Reference	–
		T	12 (2.1)	17 (2.0)	1.06 (0.50–2.24)	0.88
	Dominant	CC	271 (95.8)	407 (96.0)		
		CT + TT	12 (4.2)	17 (4.0)	1.06 (0.50–2.26)	0.88
	Co-dominant	GG	280 (98.9)	410 (96.7)	Reference	–
rs10983755		GA	3 (1.1)	14 (3.3)	0.31 (0.09–1.10)	0.06
G/A		AA	0 (0)	0 (0)	–	–
	Allelic	G	563 (99.5)	834 (98.3)	Reference	–
		A	3 (0.5)	14 (1.7)	0.32 (0.09–1.11)	0.06
	Dominant	GG	280 (98.9)	410 (96.7)		
		GA + AA	3 (1.1)	14 (3.3)	0.31 (0.09–1.10)	0.06
TLR9	Co-dominant	TT	86 (30.4)	125 (29.5)	Reference	–
rs352140		TC	134 (47.3)	204 (48.1)	0.96 (0.67–1.36)	0.80
C/T		CC	63 (22.3)	95 (22.4)	0.96 (0.63–1.47)	0.86
	Allelic	T	306 (54.1)	454 (53.5)	Reference	–
		C	260 (45.9)	394 (46.5)	0.98 (0.79–1.21)	0.85
	Dominant	TT	86 (30.4)	125 (29.5)		
		TC + CC	197 (69.6)	299 (70.5)	0.96 (0.69–1.33)	0.80
	Recessive	TC + TT	220 (77.7)	329 (77.6)		
		CC	63 (22.3)	95 (22.4)	0.99 (0.69–1.42)	0.96

SNVs Single nucleotide variants, *OR* odds ratio, *CI* Confidence interval, *SLE* Systemic lupus erythematosus

Therefore, this variant might only contribute to SLE susceptibility in specific populations.

Our study had some limitations. For example, we lacked ancestry-informative markers. The sample size was only moderate for patients with SLE. We did not evaluate other variants of both genes that could be associated with both RA and SLE in our population. The allele frequencies of the SNPs evaluated in our patients and controls were different from those reported in some populations. This difference could affect the susceptibility to, or protection against, one or both ADs. For example, the minor alleles of both *TLR4* variants, rs4986790A/G and rs4986791C/T, were found at frequencies of 2.2%, and 2.0%, respectively, in our controls. These frequencies were similar to those reported in Asians (0.6% and 4%, respectively) (6), but different from those found in Europeans (8%, and 7%, respectively) (8), and Africans (7.4% and 5.6%, respectively) [27]. Finally, the associations between polymorphisms and diseases are evaluated under the allelic and genotypic models [28]. However, the minor allele frequencies for the three *TLR4* variants were extremely low (less than 3.3%) in both the patients and controls in our population; consequently, we could not identify minor homozygous genotypes in either group, under the allelic (1 vs. 2) or genotypic (codominant; 11 vs. 12) models, the frequencies of these genetic markers were practically identical; consequently, our results suggested no association between *TLR4* and *TLR9* variants in RA or SLE.

Conclusion

Our results suggested that three *TLR4* variants were not associated with the susceptibility to SLE or RA. This conclusion was based on the evaluation of minor alleles and analyses with the codominant model (11 vs. 12), but not other genetic models, including a recessive model. Additionally,

we showed that a *TLR9* SNP was not a risk factor for RA or SLE in a Mexican population.

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Authors' contribution M.D. REB-C participated with the recruitment of patients with RA and SLE. MSc. JCL-V and PhD. ISA-U implemented the experiments, also participated with the evaluation and data analysis. PhD JR-B was responsible for the conception and design of the experiments. PhD JR-B and ISA-U contributed to the writing of the manuscript.

Declarations

Conflict of interest The authors declare that there are no personal or financial conflicts of interest regarding the present study.

Ethical approval This study was conducted according to the Declaration of Helsinki and approved by the Research and Bioethics Committee of HJM (Registry Number 0446/18-I).

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