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PAPER

TLR7/8/9 polymorphisms and their associations in systemic lupus erythematosus patients from Southern Brazil

BP dos Santos[1], JV Valverde[1], P Rohr[1], OA Monticielo[2,3], JCT Brenol[2], RM Xavier[2] and JAB Chies[1]
[1]Laboratory of Immunogenetics, Department of Genetics, Universidade Federal do Rio Grande do Sul, Brazil; [2]Division of Rheumatology, Department of Internal Medicine, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Brazil; and [3]Department of Internal Medicine, Universidade Federal de Santa Maria, Brazil

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease and can affect several organs and systems. It is characterized by high production of autoantibodies against nuclear compounds. TLR7/8/9 are responsible for nucleic acid recognition and they trigger proinflammatory responses through activation of NK-kappaB and Type I IFN production, making a bridge between the innate and the adaptative immune systems. We analyzed the frequency of *TLR7* rs179008, *TLR8* rs3764880, *TLR9* rs5743836 and rs352140 in 370 patients with SLE and 415 healthy controls from southern Brazil. All analyses were conducted with regard to gender and ethnicity. Genotypic and allelic frequencies were different for *TLR7* rs179008 (0.253 vs. 0.163, $p = 0.020$ and $p = 0.003$, OR for T allele: 1.74 CI 95% 1.12–2.70) and *TLR9* rs5743836 (0.174 vs. 0.112, $p = 0.045$ and $p = 0.017$, OR for C allele: 1.59, CI 95% 0.99–2.57) between European-derived female groups. A higher frequency was observed for the presence of Anti-SSa/Ro for *TRL9* rs5743836 C allele carriers (0.228 vs 0.126, Bonferroni corrected $p = 0.06$). No statistical differences were found for *TLR9* haplotypic analyses. We suggest that *TLR7* rs179008 and *TLR9* rs5743836 can be considered SLE susceptibility factors for women of European descent in our population. *Lupus* (2012) **21,** 302–309.

Key words: African-derived; autoimmunity; European-derived; haplotypes; nucleic acid recognition; toll-like receptor

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects many organs and systems. SLE is characterized by dysregulation in the production of antibodies, leading to high titers of autoantibodies, especially antinuclear antibodies such as anti-DNA, anti-RNA and anti-RNP. These antibodies lead to the formation and deposition of immunocomplexes, resulting in intense inflammatory response and tissue damage. Like other autoimmune diseases, SLE affects mainly women, at a ratio of 9:1, probably due to hormonal effects.[1,2] The causes of SLE are still unknown, although genetic, immunological and environmental factors are certainly involved in its development.

One aspect related to SLE pathology is Type I interferon (IFN) production.[3–5] Type I IFN is involved in typical immune responses against viruses, and it can be released by various cell types, especially antigen-presenting cells (APCs).[4,6] One way to produce high amounts of Type I IFN is through APCs when they recognize Toll-like receptor (TLR) ligands.[7] TLRs are the best-studied pattern-recognition receptors and, in the context of SLE, TLR7/8/9 stand out. TLR7 and TLR8 recognize RNA[8] and TLR9 recognizes DNA.[9] TLR7 and TLR9 are expressed in both B and plasmacytoid dendritic cells (pDC), and are involved in 95% of all Type I IFN produced. TLR8 is expressed in monocyte-derived cells, such as macrophages and myeloid DC (mDC).[10,11]

It has been suggested that the presence of high titers of autoantibodies against antinuclear antigens in patients with SLE involves excessive apoptosis. This excessive apoptosis could release the nuclear

Correspondence to: José Artur Bogo Chies, Universidade Federal do Rio Grande do Sul, Departamento de Genética, Instituto de Biociências, Av. Bento Gonçalves 9500 - Prédio 43323 - Lab. 212, CEP 91501-970, Agronomia - Porto Alegre, RS – Brasil
Email: jabchies@terra.com.br
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autoantigens, leading to the formation of immunocomplexes.[12] As soon as the immunocomplexes are internalized by APCs, RNA can be recognized by TLR7 and TLR8 and DNA by TLR9, activating a signaling pathway that leads to the release of Type I IFN and other proinflamatory cytokines. Thus, in principle, TLR function or expression dysregulation could trigger an autoimmune process. As SLE is characterized by reactivity against nucleic acids, and as TLR7/8/9 are responsible for their recognition, they are interesting study targets in SLE.

The *TLR7* gene is located on Xp22.3-p22.2. It harbors an interesting polymorphism, rs179008, which leads to the exchange of a Gln (A allele) to a Leu (T allele) at position 11 in the peptide, which according to a prediction made by Moller-Larsen and colleagues, shortens the TLR7 protein N region and extends the hydrophobic region within the signal sequence, indicating that it can affect TLR7 processing.[13] An elegant study carried out in HIV-positive patients showed that, ex vivo, the presence of the Leu variant was associated with decreased IFNα but normal IL-6 production.[14]

TLR8 is located on chromosome X, 16 kb away from *TLR7*, with little linkage disequilibrium between them.[13,15] This gene encodes two splice variants (TLR8v1 and TLR8v2) with alternative translation start sites.[16,17] Gantier and colleagues showed that the control of protein expression is fine-tuned by rs3764880, a polymorphism that leads to an A to G exchange at the first codon position, the G allele being responsible for increasing TLR8v1 translation without changes in mRNA levels or protein function.[18] Interestingly, an earlier study showed, through an overexpression assay, that the G allele leads to decreased NF-kappaB release, and this can result in a reduced activation state of the immune system, leading to a slower clinical natural course of the disease in patients with HIV.[19] The G allele was also related to protection against damage from active tuberculosis.[20]

TLR9 is located on 3p21.3. Lazarus et al. scanned the gene and a total of 20 single nucleotide polymorphisms were identified, albeit two of them are enough to distinguish the four common *TLR9* haplotypes: rs5743836 and rs352140.[21] The former is located in the promoter at the position −1237 and corresponds to a T to C exchange, creating a potential binding site for NF-kappaB.[22] This polymorphism has been implicated in chronic inflammatory diseases including asthma[21] and Crohn's Disease.[23] The rs352140 polymorphism is located at position +2848 in the exon 2, corresponds to a G to A exchange, and does not alter the amino acid sequence. The AA genotype has already been associated with high TLR9 expression and intracellular IgM in B cells in patients with primary biliary cirrhosis,[24] but to our knowledge there are no studies reporting functional assays.

Considering the inflammatory status and the high levels of antinuclear autoantibodies in patients with SLE, and considering the role of TLR7/8 and 9 in immune system activation, the present study aims to analyze the frequency of the polymorphisms rs179008 in *TLR7*, rs3764880 in *TLR8*, rs5743836 and rs352140 in *TLR9* among patients with SLE and healthy controls from southern Brazil, looking for a possible association of these variants with clinical and laboratory expression of the disease. A possible participation of *TLR7* rs179008, *TLR9* rs5743836 and a bias of the latter with the presence of anti-SSa/Ro in a European-derived population of female SLE patients will be presented and discussed here.

Materials and methods

Study population

The study population comprised 370 SLE patients: 342 (92.4%) women and 28 (7.6%) men; 282 (76.2%) identified as European derived and 88 (23.8%) as African derived. This classification was based on physical appearance, as judged by the researcher at the time of blood collection, and data about the ethnicity of parents/grandparents which were reported by the participants. Issues regarding the skin color-based classification criteria that is used in Brazil are well documented[25] and have been already assessed by our group in previous studies.[26,27] Also, a recent study assessing individual interethnic admixture and population substructure by means of a panel composed of 48 insertion–deletion ancestry-informative markers has validated this classification in European-derived individuals from our region.[28] The patients received follow-up care at the Division of Rheumatology of the Hospital de Clínicas de Porto Alegre. All patients fulfilled the American College of Rheumatology revised criteria for the classification of SLE.[29]

Clinical manifestations of SLE included the presence of photosensitivity, malar rash, discoid rash, oral or nasal ulcers, arthritis, serositis (pleuritis or pericarditis), nephritis and neurological diseases defined as seizures or psychosis. The laboratory evaluation included the presence of hematological disorders (hemolytic anemia, leukopenia, lymphopenia or

thrombocytopenia), positive antinuclear antibody (titer > 1:100), or other autoantibodies such as anti-dsDNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti- La/SS-B, anticardiolipin, lupus anticoagulant and false positive VDRL. The patients were also evaluated with regard to secondary antiphospholipid syndrome and Sjogren's syndrome, according to the classification criteria for both diseases,[30,31] and SLEDAI[32] and SLICC damage index[33] were applied to each patient as a measurement of disease activity and cumulative damage, respectively.

The control group was composed of 415 healthy people from the same urban center: 191 (46%) were women, 224 (54%) men; 309 (74.4%) individuals are European derived and 106 (25.6%) African derived. The study protocol was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre and informed consent according to the Declaration of Helsinki was obtained from all patients.

Genotyping

DNA was isolated using salting-out method[34] and stored at -20°C. The polymorphisms *TLR7* rs179008, *TLR8* rs3764880 and *TLR9* rs352140 were amplified using the protocol as described as Cheng and colleagues.[35] To genotype, the restriction endonucleases *Apo*I, *Nla*III and *BstU*I, respectively, were used. After treatment with restriction endonucleases, *TLR7* rs179008 and *TLR9* rs352140 cleavages were visualized in 6% polyacrylamide gel and *TLR8* rs3764880 was visualized in 8% polyacrylamide gel, and all of them were stained with silver nitrate. *TLR9* rs5743836 was genotyped as described by Carvalho and colleagues[36] and visualized in 1.5% agarose gel stained with SYBR gold®.

Statistical analysis

A descriptive analysis of data through calculation of mean and standard deviation for quantitative variables was performed, while the frequency and percentage were calculated for categorical data. We used the chi-square test or Fisher's exact test in the comparison between the presence and absence of polymorphic variants. Besides these tests, we calculated the odds ratio (OR) and confidence intervals (CI). For the comparison of clinical and laboratory variables with the presence or absence of polymorphic variants, we used the chi-square test with Bonferroni correction for the comparisons including clinical symptoms. The Hardy–Weinberg equilibrium test was performed in cases and controls using the chi-square test. Haplotype frequencies were estimated using MLocus. Data were analyzed with SPSS 15.0 and WinPEPI version 11.1. A two-tailed value of $p < 0.05$ was taken to indicate statistical significance.

Results

All analyses were performed with groups subdivided according to gender and ethnic origins since *TLR7* and *TLR8* genes are located on the X chromosome, and since the literature already reports different allelic frequencies among European and African-derived populations for some of the analyzed variants. All control groups were in Hardy–Weinberg Equilibrium, although the *TLR7* and *TLR8* genotypic frequencies did not reach equilibrium among the European-derived SLE women.

Allelic and genotypic analyses

Table 1 shows genotypic and allelic frequencies in European-derived individuals. When we compared female European-derived patients with controls, we noticed that *TLR7* rs179008 genotypic ($p = 0.020$) and allelic frequencies were different (0.253 vs.

Table 1 Genotypic and allelic frequencies in European-derived patients and controls

	Genotype			*Allele*
TLR7	**AA**	**AT**	**TT**	**T**
♀ Patients ($n = 259$)	**151 [0.583]**[a]	**85 [0.328]**[a]	**23 [0.089]**[a]	**0.253**[b]
♀ Controls ($n = 144$)	**102 [0.708]**[c]	**37 [0.257]**[c]	**5 [0.035]**[c]	**0.163**[d]
	A		**T**	
♂ Patients ($n = 23$)	18 [0.783]		5 [0.217]	0.217
♂ Controls ($n = 159$)	136 [0.855]		23 [0.145]	0.145
TLR8	**AA**	**AG**	**GG**	**G**
♀ Patients ($n = 257$)	107 [0.416]	100 [0.389]	50 [0.195]	0.389
♀ Controls ($n = 146$)	69 [0.473]	56 [0.383]	21 [0.144]	0.336
	A		**G**	
♂ Patients ($n = 23$)	16 [0.696]		7 [0.304]	0.304
♂ Controls ($n = 149$)	97 [0.651]		57 [0.349]	0.349
***TLR9* T-1237C**	**TT**	**TC**	**CC**	**C**
♀ Patients ($n = 258$)	**179 [0.694]**[e]	**68 [0.263]**[e]	**11 [0.043]**[e]	**0.174**[f]
♀ Controls ($n = 147$)	**115 [0.782]**[g]	**31 [0.211]**[g]	**1 [0.007]**[g]	**0.112**[h]
♂ Patients ($n = 23$)	16 [0.696]	6 [0.261]	1 [0.043]	0.174
♂ Controls ($n = 162$)	112 [0.691]	45 [0.278]	5 [0.031]	0.170
***TLR9* G2848A**	**GG**	**GA**	**AA**	**A**
♀ Patients ($n = 257$)	67 [0.261]	115 [0.447]	75 [0.292]	0.516
♀ Controls ($n = 145$)	38 [0.262]	76 [0.524]	31 [0.214]	0.476
♂ Patients ($n = 23$)	3 [0.130]	13 [0.565]	7 [0.305]	0.587
♂ Controls ($n = 162$)	36 [0.222]	75 [0.463]	51 [0.315]	0.546

Absolute frequency and [relative frequency] are shown for genotypes
a × c (χ^2): $p = 0.020$, b × d (Fisher): $p = 0.003$
e × g (χ^2): $p = 0.045$, f × h (Fisher): $p = 0.017$

Table 2 Genotypic and allelic frequencies in African-derived patients and controls

	Genotype			*Allele*
TLR7	**AA**	**AT**	**TT**	**T**
♀ Patients ($n=83$)	59 [0.711]	19 [0.229]	5 [0.060]	0.175
♀ Controls ($n=43$)	29 [0.674]	10 [0.233]	4 [0.093]	0.326
	A		**T**	
♂ Patients ($n=5$)	5 [1.000]		0 [0.000]	0.000
♂ Controls ($n=61$)	52 [0.852]		9 [0.148]	0.148
TLR8	**AA**	**AG**	**GG**	**G**
♀ Patients ($n=83$)	38 [0.458]	33 [0.398]	12 [0.144]	0.343
♀ Controls ($n=44$)	21 [0.477]	17 [0.387]	6 [0.136]	0.330
	A		**G**	
♂ Patients ($n=5$)	4 [0.800]		1 [0.200]	0.200
♂ Controls ($n=59$)	43 [0.729]		16 [0.271]	0.271
***TLR9* T-1237C**	**TT**	**TC**	**CC**	**C**
♀ Patients ($n=83$)	46 [0.555]	29 [0.349]	8 [0.096]	0.271
♀ Controls ($n=43$)	25 [0.581]	18 [0.419]	0 [0.000]	0.209
♂ Patients ($n=5$)	4 [0.800]	1 [0.200]	0 [0.000]	0.100
♂ Controls ($n=57$)	22 [0.386]	30 [0.526]	5 [0.088]	0.351
***TLR9* G2848A**	**GG**	**GA**	**AA**	**A**
♀ Patients ($n=83$)	33 [0.387]	37 [0.446]	13 [0.157]	0.380
♀ Controls ($n=40$)	16 [0.400]	17 [0.425]	7 [0.175]	0.388
♂ Patients ($n=5$)	1 [0.200]	3 [0.600]	1 [0.200]	0.500
♂ Controls ($n=62$)	26 [0.419]	30 [0.484]	6 [0.097]	0.339

Absolute frequency and [relative frequency] are shown for genotypes.

0.163, $p=0.003$). In the genotypic frequency analysis, the AA genotype was less represented while TT was overrepresented among patients compared with controls ($p=0.013$ and $p=0.041$, respectively). The overall OR for T allele carriers was 1.74 with a 95% CI 1.12–2.70. For *TLR9* rs5743836, female European-derived patients and controls also showed different genotypic ($p=0.045$) and allelic frequencies (0.174 vs. 0.112, $p=0.017$). In the genotypic analysis, the CC genotype was overrepresented among patients when compared with controls (0.045 vs. 0.007, $p=0.041$). The OR for the C allele was 1.59 with 95% CI 0.99–2.57.

Table 2 shows genotypic and allelic frequencies in African-derived individuals. There were no statistical differences for either genotypic or allelic frequencies between groups.

Haplotype analyses

Since two polymorphisms were analyzed in *TLR9*, rs5743836 in promoter and rs352140 in exon 2, we estimated haplotype frequencies (Table 3). Comparing patients and controls, no statistical differences were observed in haplotype frequencies, although a bias was observed between female European-derived patients and controls ($p=0.082$). Differences due to the ethnic origin of individuals become quite evident when European-derived and African-derived groups are compared. For instance, African-derived individuals have a higher overall CG haplotype frequency than European-derived individuals.

Table 3 Haplotype estimated frequencies and number of chromosomes (n) in European- and African-Derived Individuals

	Haplotype		*European-derived*		*African-derived*	
	T-1237C	***G2848A***	*freq*	*n*	*freq*	*n*
♀ Patients	T	G	0.450801	234[a]	0.472895	79[e]
($n=518/166$)	T	A	0.375198	194[a]	0.256021	42[e]
	C	G	0.032013	17[a]	0.147587	24[e]
	C	A	0.141988	73[a]	0.123497	21[e]
♀ Controls	T	G	0.511698	150[b]	0.485093	42[f]
($n=294/86$)	T	A	0.376057	111[b]	0.305605	26[f]
	C	G	0.013151	4[b]	0.127476	11[f]
	C	A	0.099094	29[b]	0.081826	7[f]
♂ Patients	T	G	0.413043	19[c]	0.500000	5[g]
($n=46/10$)	T	A	0.413044	19[c]	0.400000	4[g]
	C	G	0.000000	0[c]	0.000000	0[g]
	C	A	0.173913	8[c]	0.100000	1[g]
♂ Controls	T	G	0.424313	138[d]	0.463610	59[h]
($n=326/128$)	T	A	0.403908	132[d]	0.185173	24[h]
	C	G	0.026607	9[d]	0.196942	25[h]
	C	A	0.145172	47[d]	0.154275	20[h]

a × b (χ^2): $p=0.082$
c × d (Fisher): $p=0.870$
e × f (χ^2): $p=0.641$
a × e (χ^2): $p=0.00000054$
residual: TA: $p=0.004$, CG: $p=0.00000012$
b × f (χ^2): $p=0.000034$
residual: CG: $p=0.0000017$
d × h (χ^2): $p=0.00000000027$
residual: TA: $p=0.000011$, CG: $p=0.000000001$

Discussion

TLR7/8/9 are nucleic acid receptors involved in NF-kappaB activation and in the induction of Type I IFN. These receptors play an important role in activation and regulation of DC and B cells, which are responsible for pathogen clearance, antigen recognition and antibody production, critical findings in SLE.

There is evidence indicating that TLR7 is involved in the development of autoimmunity. Studies in congenic mice bearing the Y-linked autoimmune accelerator (yaa) lupus susceptibility locus have shown that differences in *TLR7* expression as well as in environmental factors that induce TLR7 responses may result in increased B-cell sensitivity to RNA-containing autoantigens.[37,38] Also, transgenic mice with a twofold increased *TLR7*

expression showed increased production of RNA-related autoantibodies and spontaneously developed autoimmunity.[39] Nevertheless, in humans, association studies with *TLR7* remain controversial: *TLR7* rs3853839 (3'UTR localization) was found to be associated with SLE in Chinese and Japanese populations, with a stronger effect in men when compared with women.[40] Recently, Kawasaki and colleagues evaluated Japanese women with SLE and reproduced the previous association of rs3853839. They also observed associations of two other polymorphisms with SLE: rs179019 and rs179010, supporting the participation of *TLR7* in SLE among Asian populations.[41] In contrast, a study carried out in Spanish populations did not associate the *TLR7* rs179008 polymorphism with SLE susceptibility.[42]

In the present study, we investigated a putative functional polymorphism in *TLR7* in patients with SLE and ethnically matched controls from the southernmost state of Brazil. Our results revealed an increased frequency of the T allele among European-derived female patients suggesting, for the first time, this variant as a susceptibility factor in SLE. As shown in Table 1, the T allele was over-represented in European-derived women (0.253 vs. 0.163; $p = 0.003$), resulting in OR 1.74 (CI 95% 1.12–2.70) for T carriers, or OR 3.11 (CI 95% 1.19–9.42) for TT vs. AA carriers. The T allele has already been associated with higher susceptibility to HCV infection and reduced response to an IFNα-based therapy in chronically HCV-infected German women.[43] Furthermore, in a study performed by Oh and colleagues with HIV patients, the same variant was associated with higher viral loads, accelerated progression to advanced immune suppression, increased susceptibility to HIV-1 in women and decreased IFNα production after stimulation of peripheral blood mononuclear cells with imiquinod, a TLR7 ligand.[14]

Considering *TLR8*, it is believed that this gene encodes two splice variants with alternative translation start sites.[16,17] A recent study showed that rs3764880 fine-tunes translation of these two main isoforms in monocytes, and that TLR8's biochemical function is independent of the N-terminus region.[18] Concerning its relationship with SLE, there are few studies assessing *TLR8*[44,45] but none evaluates its polymorphisms in this disease. Our study is the first to study *TLR8* within SLE, and our results did not find an association between rs3764880 and SLE susceptibility or clinical symptoms. Nevertheless, these negative results do not rule out *TLR8* as a candidate gene in SLE since it plays important roles in the regulation of monocyte-derived cells, and the function and role of the translated TLR8 isoforms have not been not well elucidated.

It is important to remember that Hemmi et al. showed that *TLR9* recognizes bacterial DNA through CpG motifs,[46] and that this understanding remained current until years later when Haas and colleagues showed that the DNA sugar backbone 2' deoxyribose represents a prime determinant for ssDNA–TLR9 interactions.[9] This means that self-DNA can act as a TLR9 ligand, suggesting this molecule as potentially relevant in SLE. We evaluated two polymorphisms in *TLR9*: rs5743836 in the promoter region, and rs352140 in exon 2. The former showed differences in genotypic and allelic frequencies between female European-derived patients and matched controls ($p = 0.045$ and $p = 0.017$, respectively), suggesting the C allele as a susceptibility factor in SLE (OR 1.59 CI 95% 0.99–2.57 for C allele carriers). However, studies in SLE patients with a European-derived or European genetic background did not find this association,[47,48] while studies in Japanese, Korean and Chinese samples did not perform any analysis because of the low C allele frequency in these populations.[49,51]

When clinical symptoms were analyzed, those patients who present anti-SSa/Ro ($n = 92$) showed increased frequency of CC genotype compared with those who do not present anti-SSa/Ro ($n = 143$) ($p = 0.005$ for general comparison, and $p = 0.003$ revealing CC genotype increased in anti-SSa/Ro positive). The overall OR for C allele carriers was 1.83 with CI 95% 1.03–3.22. Nevertheless, this finding lost statistical significance after Bonferroni correction ($p = 0.06$), showing the need for a larger sample. For the exonic polymorphism rs352140 no associations were observed, either between groups or according to clinical symptoms.

The functional consequences of the rs5743836 C allele have been discussed in the literature. Novak and colleagues showed a significantly higher promoter activity in the TT allelic variant sequence compared with the CC allelic variant sequence ($p = 0.018$).[52] In this case, we can imagine that both variants – T for *TLR7* rs179008, and C for *TLR9* rs5743836 – could play an important role in initial SLE susceptibility, where carriers could have increased susceptibility to viral infections, for example, which are largely related to SLE,[53–59] and consequently become more prone to SLE. Nevertheless, results from Ng and colleagues are not in agreement with those from Novak et al.[60] It was already known that the C allele creates a potential binding site for NF-kappaB, and

Table 4 *TLR9* rs5743836 and rs352140 haplotype frequencies worldwide

Haplotype		*Lazaurus et al. (2003)*[21]			*Lammers et al. (2005)*[61]	*Ito et al. (2007)*[62]	*Berghofer et al. (2005)*[63]	*Our data*	
rs5743836	*rs352140*	*African american*	*European american*	*Hispanic american*	*Italian*	*Japanese*	*German*	*Euro-derived*	*Afro-derived*
T	G	0.444	0.435	0.396	0.400	0.5196	0.4400	0.4651	0.4670
T	A	0.408	0.413	0.562	0.440	0.4657	0.4221	0.3914	0.2369
C	G	0.056	0.000	0.000	0.010	0.0147	0.0049	0.0207	0.1761
C	A	0.092	0.152	0.042	0.150	0.0000	0.1332	0.1228	0.1200
Total of individuals		24	23	24	224	102	102	310	110

these authors showed that the C allele has higher promoter activity in response to activators of the NF-kappaB pathway ($p \leq 0.001$). Therefore, we can hypothesize that, when activated, NF-kappaB can bind to the *TLR9* promoter, enhancing its expression and leaving more TLR9 available in endosomes. Thus, in individuals with SLE, less host DNA or other ligand would be necessary to activate pDC and B cells in a TLR9 manner.

We analyzed *TLR9* haplotypes according to gender and ethnicity (Table 3) and no significant frequency deviations between patients and controls were observed. Nevetheless, differences among individuals from distinct ethnic origins become evident. For instance, African-derived individuals have an overall CG haplotype frequency higher than European-derived individuals. Indeed, a previous study had already suggested a higher frequency of the CG haplotype among African-derived individuals, despite considering a small sample ($n = 24$).[21] The haplotype frequencies of rs5743836 and rs352140 from different human populations can be seen in Table 4. These data reinforce the fact that gene/disease association studies should take into account the genetic/ethnic background of the patients.

In conclusion, we analyzed *TLR7/8/9* polymorphisms in patients with SLE from southern Brazil. Genotypic and allelic frequencies were significantly different for *TLR7* rs179008 ($p = 0.020$ and $p = 0.003$, OR for T allele: 1.74 CI 95% 1.12–2.70) and *TLR9* rs5743836 ($p = 0.045$ and $p = 0.017$, OR for C allele: 1.59 CI 95% 0.99–2.57) comparing female European-derived SLE and control groups from southern Brazil. Therefore, we suggest that these variants may be involved in SLE susceptibility in European-derived women.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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