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Toll-like receptor 5 and Toll-like receptor 9 single nucleotide polymorphisms and risk of systemic lupus erythematosus and nephritis in Egyptian patients

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Abstract

Background: Toll-like (TLRs) play a crucial role in both adaptive and innate immunity. The aim of the present study was to assess the association of TLR5-rs5744168, TLR9-rs187084, and TLR9-rs352140 single nucleotide polymorphisms (SNPs) with susceptibility to systemic lupus erythematosus (SLE) and lupus nephritis (LN) in Egyptian patients.

Results: The C allele and homozygous CC genotype of the TLR9-rs352140 in co-dominant and recessive models were more prevalent in SLE patients than controls ($P = 0.047$, $P = 0.017$, and $P = 0.005$ respectively). In contrast, allelic and genotyping distribution of TLR5-rs5744168 and TLR9-rs187084 SNPs showed no association with the risk of SLE. The T allele of the TLR5-rs5744168 was more prevalent in LN patients than controls ($P = 0.021$). The homozygous TT genotype of TLR5-rs5744168 SNP was more prevalent in LN patients in the co-dominant and the recessive models than controls ($P = 0.036$ and $P = 0.011$ respectively). The C allele of the TLR9-rs352140 was more prevalent in LN patients than controls ($P = 0.015$). The homozygous CC genotype of the TLR9-rs352140 SNP was more prevalent in LN than controls in co-dominant and recessive models ($P = 0.002$ and $P < 0.001$). In the recessive model of the TLR5-rs5744168 SNP, the TT genotype was found in 3.2% of the SLE patients while none of the SLE patients without LN or controls had TT genotype ($P = 0.036$). Also, in the recessive model of the TLR9-rs352140 SNP, the CC genotype was significantly more frequent in SLE patients with LN than without LN (44.4% vs 29.9%, $P = 0.045$).

Conclusion: Our results support the potential role of TLR5-rs5744168 SNP and TLR9-rs3532140 SNP not only in increasing the risk for development of SLE, but also in increasing the risk of LN in SLE patients among the Egyptian population.

Keywords: Toll-like receptor, Systemic lupus erythematosus, Lupus nephritis, Single nucleotide polymorphisms

Background

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by autoantibody production and formation of immune complexes. Renal involvement is prevalent among SLE patients, affecting ~50% of the patients. Lupus nephritis (LN) is a major risk factor for overall morbidity and mortality in SLE [1]. The pathogenesis of SLE and lupus nephritis (LN) is highly complex. A complex interaction between the genetic predisposition and environmental factors leads to the breakdown of tolerance to self-antigens, production of pro-inflammatory cytokines, autoantibodies, and complement deficiencies, all of which are implicated in the development of SLE [2].

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Aberrations involving innate immune mechanisms had been implicated in the amplification of the inflammatory response and had a pivotal role in the pathogenesis of SLE [3]. Toll-like receptors (TLRs), a key component of the innate immune system, are widely expressed in innate immune cells and resident cells of various organs, including kidneys [4]. TLRs can recognize self-antigens, and accordingly, TLRs may be involved in the initiation of autoreactivity and the amplification of tissue damage in autoimmune conditions [5]. Genetic variation affecting TLR signaling is, therefore, expected to affect the susceptibility to autoimmune diseases including SLE and LN [6–8].

Linkage studies have identified a major SLE susceptibility locus to human chromosome 1q41, where TLR5 resides [9, 10]. TLR5 is expressed in many cells including the dendritic cells. TLR5 recognizes bacterial flagellin and acts as a receptor to capture flagellated bacteria. Flagellin is a potent immune activator, and signaling of flagellin via TLR5 evokes both innate inflammatory responses and the development of adaptive immunity [11]. A stop codon polymorphism within the ligand-binding domain was shown to decrease flagellin signaling [12]. Given the critical role of TLR5 in inflammatory signaling pathways and the linkage studies that have mapped a major SLE susceptibility locus where TLR5 resides [9], TLR5 has been considered a potential candidate for increased susceptibility to SLE. Though, it had been reported that TLR5 stop codon polymorphism is associated with decreased SLE susceptibility [13].

The TLR9 gene (chromosome 3p21.3) is residing one of the defined susceptibility regions for SLE [14]. TLR9 ligation is followed by type I interferon production in immune cells especially dendritic cells [15]. TLR9 is an important costimulatory molecule for both B cells and dendritic cells that respond to chromatin immune complexes [16]. TLR9-dependent type I interferons promote the responsiveness to B-cell receptor crosslinking in B cells and render dendritic cells responsive to endogenous nucleic acids after upregulation of TLR9 and, thus, initiate autoimmunity [17]. The rs351240 variant is located in the second exon, the major coding region of the gene [18] while the rs187084 variant is located in the promoter region, where it can regulate TLR9 expression [19]. Both variants were among the most studied single nucleotide polymorphisms (SNPs) in the TLR9 gene with SLE and LN risk; however, the studies had provided controversial results [16, 20–22]. A previous study had provided strong evidence supporting the independent role of TLR9 in the pathogenesis of SLE and LN [23]. Conversely, deletion of TLR9 in lupus-prone mice models did not result in disease remission, as predicted, but to flare of the disease, proposing a protective role of TLR-9 against SLE in mice [24, 25].

The aim of this study was to assess the association of TLR5-rs5744168, TLR9-rs187084, and TLR9-rs352140 SNPs with increased susceptibility to SLE and risk of LN in Egyptian patients.

Methods

Patients and controls

A total of 200 patients (177 females and 23 males) with SLE were recruited in the period from January 2017 to January 2019 for participation in the present study. All patients fulfilled the revised criteria of the American College of Rheumatology for SLE [26]. Among the SLE patients, 63 patients had biopsy-proven LN. The study included 200 (173 females and 27 males) age- and sex-matched healthy volunteers unrelated to patients to serve as a control group. The exclusion criteria were (i) younger than 18 years; (ii) presence of diseases such as diabetes mellitus, hypertension, overlap syndrome, or neoplastic disorders; (iii) presence of infections such as chronic hepatitis C or B viruses or HIV; and (iv) patients with renal disease due to causes other than the current SLE. A written consent was obtained from all eligible participants before enrolment into the study. The study was approved by the local ethical committee.

Sample size

Based on data from literature [27], to calculate the sample size with precision/absolute error of 5% and type 1 error of 5%, the following formula is use: Sample size $= [(Z_{1-\alpha/2})^2 \cdot P(1-P)]/d^2$. Therefore, sample size $= [(1.96)^2 \cdot (0.345) \cdot (1-0.345)]/(0.066)^2 = 199.3$. Based on the above formula, the sample size required for the study is 200 SLE patients and 200 non-SLE controls.

Genotyping of TLR genes

Five milliliters of venous blood was obtained from every participant for genotyping of TLR5-rs5744168 and TLR9-rs187084 and rs352140 SNPs. DNA was isolated from peripheral leucocytes using G-spin^{TM} Total DNA Extraction Mini Kit (Intron Biotechnology, Korea, Cat. no. 17045). Genotyping technique was based on detection of SNPs through polymerase chain reaction followed by restriction fragment-length polymorphism.

Identification of TLR5-rs5744168 SNP

Identification of SNP of rs5744168 variant of TLR5 gene (also known as Arg392Stop or C1174T) was conducted by employing forward primer: TAC GGA CTT GAC AAC CTC CA and reverse primer: TGG ATG AGG TTC GCT GTA AGG at Chromosome 1, promoter region. PCR products were digested using DdeI

restriction endonuclease for TLR5-rs5744168. The products of DNA digestion were fractionated on 2% agarose gel using 50-bp DNA ladder markers and visualized using the UV light. The observed patterns of polymorphism for TLR5-rs5744168 polymorphism were 83 bp and 207 bp for the C allele or 83 bp, 94 bp, and 113 bp for the T allele.

Identification of TLR9-rs187084 SNP

Identification of TLR9 gene polymorphism for SNP for rs187084 polymorphic variant was conducted by employing forward primer: CCT GCC TGC CAT GAT ACC AC and reverse primer: TGC TAG CAC ACC GGA TCA TT at Chromosome 3, promoter region. PCR products were digested using AflII restriction for TLR9-rs187084. Then, they were fractionated on 2% agarose gel using 50-bp DNA ladder markers and visualized using the UV light. The observed patterns of polymorphism for TLR9-rs187084 were 242 bp and 79 bp for the T allele and 321 bp for the C allele.

Identification of TLR9-rs352140 SNP

Identification of TLR9 gene polymorphism for SNP for rs352140 polymorphic variant was conducted by employing forward primer: GCA GCA CCC TCA ACT TCA CC and reverse primer: GGC TGT GGA TGT TGT TGT GG at Chromosome 3, exon 2. PCR products were digested with BstUI restriction enzyme at 37 °C for 3 h. The BstUI digestive products were run by 2% agarose gel electrophoresis for 30 min and stained with ethidium bromide, and the bands were visualized under UV light. Digested PCR products yielded 360-bp bands in TT homozygotes, 133- and 227-bp bands in CC homozygotes, and all 3 bands in CT heterozygotes.

To ensure reproducibility, genotyping was replicated on a random 10% of the samples, and the results were 100% consistent.

Statistical analysis

All statistical calculations were done through SPSS version 20.0 statistic software. All continuous variables were normally distributed and were presented in mean ± standard deviation (*SD*). Categorical variables were presented in number and percentage. The comparisons were performed using independent sample Student's *t* test for comparison between two continuous variables. A chi-square test was used for comparison of categorical variables. Odds ratio was calculated to measure the association between an exposure (presence of alleles) and an outcome (occurrence of SLE or LN). The 95% confidence interval (CI) was used to estimate precision of *OR*. The Hardy-Weinberg equilibrium (HWE) was evaluated by the goodness-of-fit X^2 test to compare the observed genotype frequencies with the expected frequencies in controls in order to test the assumption that genotype frequencies in a population will remain constant from generation to generation [28]. Statistical significance was set at $P \leq 0.05$.

Results

The study included 200 lupus patients (177 females and 23 males) and 200 controls (173 females and 27 males). The ages of the SLE patients ranged from 22 to 45 years with mean age of 33.6 ±7.2 years, and the ages of the controls ranged from 21 to 45 years with mean age of 32.9 ±7.1 years. The two groups did not differ significantly regarding the age and sex ($P = 0.398$ and $P = 0.545$ respectively).

In the present study, genotyping frequencies of TLR5 gene SNP rs5744168, TLR9 gene SNP rs187084, and

Table 1 Comparison of allele distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between SLE patients and controls

	SLE patients		Controls		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
C allele [R]	387	96.8	393	98.3			
T allele	13	3.3	7	1.8	1.846	0.174	1.89 [0.75–4.78]
TLR9-rs187084 SNP							
C allele [R]	167	41.8	179	44.8			
T allele	233	58.3	221	55.3	0.733	0.392	1.130 [0.85–1.49]
TLR9-rs352140 SNP							
C allele	223	55.8	195	48.8			
T allele [R]	177	44.3	205	51.3	3.928	0.047*	1.32 [1.00–1.75]

The chi-square test is used. *P* is set significant if ≤ 0.05, significant values are marked by *

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *SLE* Systemic lupus erythematosus, *OR* Odds ratio, *CI* Confidence interval, *R* Reference allele

TLR-9 rs352140 SNP fitted the HWE among controls (*P*=0.064, *P*=0.344, and 0.998 respectively).

Comparison of the investigated SNPs between SLE patients and controls

The allelic distribution revealed that the C allele of the TLR9-rs3532140 is more prevalent in SLE patients than controls (55.8% vs 48.8%). This difference was significant (*P*= 0.047, *OR*= 1.32, *95% CI*=1.00–1.75) (Table 1).

In addition, the homozygous CC genotype of the TLR9-rs3532140 is significantly more prevalent in SLE patients in comparison to the controls in the co-dominant model (*P*= 0.017) as well as in the recessive model (*P* = 0.005) (Table 2).

In contrast, allelic and genotyping distribution of TLR-5-rs5744168 SNP and TLR9 rs187084 SNP showed no association with risk SLE (Tables 1 and 2).

Comparison of the investigated SNPs between SLE patients with LN and controls

Allelic distribution of the TLR5-rs5744168 revealed that the T allele is more prevalent in LN patients in comparison to the controls (5.6% and 1.8% respectively). This

Table 2 Comparison of genotyping distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between SLE patients and controls

	SLE patients		Controls		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
Co-dominant model							
CC	189	94.5	193	96.5			
CT	9	4.5	7	3.5			
TT	2	1	0	0	2.292	0.318	
Recessive model							
CC+CT [R]	198	99	200	100			
TT	2	1	0	0	2.010	0.156	5.05 [0.24–105.87]
Dominant model							
CC [R]	189	94.5	193	96.5			
CT+TT	11	5.5	7	3.5	0.931	0.335	1.60 [0.61–4.23]
TLR9-rs187084 SNP							
Co-dominant model							
CC	29	14.5	38	19			
CT	109	54.5	103	51.5			
TT	62	31	59	29.5	1.453	0.484	
Recessive model							
CC+CT [R]	138	69	141	70.5			
TT	62	31	59	29.5	0.107	0.744	1.07 [0.70–1.65]
Dominant model							
CC [R]	29	14.5	38	19			
CT+TT	171	85.5	162	81	1.452	0.228	1.38 [0.82–2.35]
TLR9-rs352140 SNP							
Co-dominant model							
CC	69	34.5	44	22.0			
CT	85	42.5	107	53.5			
TT	46	23.0	49	24.5	8.147	0.017*	
Dominant model							
CC+CT	154	77.0	151	75.5			
TT [R]	46	23.0	49	24.5	0.124	0.725	1.09 [0.69–1.72]
Recessive model							
CC	69	34.5	44	22.0			
CT+TT [R]	131	65.5	156	78.0	7.709	0.005*	1.87 [1.20–2.91]

The chi-square test is used. *P* is set significant if $\leq$ 0.05, significant values are marked by *

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *SLE* Systemic lupus erythematosus, *OR* Odds ratio, *CI* Confidence interval, *R* Reference genotype

difference was significant (P= 0.021, OR=3.30, *95% CI* = 1.14–9.60). Regarding the TLR9-rs3532140 variant, the C allele was more prevalent in the LN patients in comparison to the controls (61.1% vs 48.8%). This difference was significant (P= 0.015, OR= 1.65, *95% CI* = 1.10–2.49) (Table 3).

In addition, 3.2% of the LN patients, but none of the controls, showed homozygous TT genotype of TLR5-rs5744168 SNP. The distribution of homozygous TT genotype of TLR5-rs5744168 SNP associated with increased risk for LN in the co-dominant model (P= 0.036) and in the recessive model (P= 0.011). Regarding the TLR-9 rs352140 SNP, the homozygous CC genotype is more frequent in LN (44.4%) than in controls (22%). This difference was significant in the co-dominant model (P= 0.002) and in the recessive model (P<0.001) (Table 4).

However, allelic distribution and genotyping distribution of TLR9-rs187084 SNP showed no association with the risk of LN (Tables 3 and 4).

Comparison of the investigated SNPs between SLE patients with and without LN

The difference in frequency of T allele of the TLR5-rs5744168 SNP between the SLE patients without LN compared to those with LN approached but did not reach significance level (2.2% vs 5.6% respectively, P=0.078) (Table 5). However, in the recessive model of the TLR5-rs5744168 SNP, the TT genotype was found in 3.2% of the SLE patients while none of the SLE patients without LN or controls had the TT genotype (P=0.036). Also, in the recessive model of the TLR9-rs352140 SNP, the CC genotype was significantly more frequent in SLE patients with LN than without LN (44.4% vs 29.9%, P=0.045) (Table 6).

Discussion

A major finding of the present study is that the T allele of the TLR5-rs5744168 SNP is significantly more frequent in SLE patients with LN compared to the controls. Also, the T allele of the TLR5-rs5744168 SNP is significantly more frequent in SLE patients with LN compared to those without LN; however, difference in frequency between the SLE patients without LN compared to those with LN approached but did not reach significance level. In the recessive model of the TLR5-rs5744168 SNP, the TT genotype was found in 3.2% of the SLE patients with LN while none of the SLE patients without LN or controls had the TT genotype.

The rs5744168 SNP of TLR5, involved in cytosine-to-thymidine transition at base pair 1174, generates a stop codon that could affect TLR5 function [29]. In the present study, and despite the TLR-5 gene location at the 1q41–1q42 region, known as a major susceptibility locus to SLE [9, 10, 30], we found no evidence to support a significant association between the TLR5-rs5744168 variant and susceptibility to or protection from SLE. This finding comes in agreement with other case-control studies, showing that this polymorphism did not influence susceptibility to SLE. The study by Demirci et al. [31] found no statistically significant relationship between TLR5 and SLE risk. Another study from China reported that TLR5-rs5744168 gene polymorphism was unrelated with SLE susceptibility in Chinese patients [32]. Similarly, no association was observed between the TLR5-rs5744168 polymorphism and SLE susceptibility in the European population [33]. In the Spanish population, no statistically significant differences were found when the allele and genotype distribution of TLR5-rs5744168 polymorphism was compared between SLE patients and controls [34].

Table 3 Comparison of allele distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between LN patients and controls

	SLE patients with LN		Controls		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
C allele [R]	119	94.4	393	98.3			
T allele	7	5.6	7	1.8	5.356	0.021*	3.30 [1.14–9.60]
TLR9-rs187084 SNP							
C allele [R]	48	38.1	179	44.8			
T allele	78	61.9	221	55.3	1.73	0.188	1.29 [0.86–1.94]
TLR9-rs352140 SNP							
C allele	77	61.1	195	48.8			
T allele [R]	49	38.9	205	51.3	5.863	0.015*	1.65 [1.10–2.49]

The chi-square test is used. *P* is set significant if ≤ 0.05, significant values are marked by *

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *LN* Lupus nephritis, *OR* Odds ratio, *CI* Confidence interval, *R* Reference allele

Table 4 Comparison of genotyping distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between LN patients and controls

	SLE patients with LN		Controls		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
Co-dominant model							
CC	58	92.1	193	96.5			
CT	3	4.8	7	3.5			
TT	2	3.2	0	0	6.649	0.036*	
Recessive model							
CC+CT [R]	61	96.8	200	100			
TT	2	3.2	0	0	6.398	0.011*	16.30 [0.77–344.14]
Dominant model							
CC [R]	58	92.1	193	96.5			
CT+TT	5	7.9	7	3.5	2.165	0.141	2.38 [0.73–7.77]
TLR9-rs187084 SNP							
Co-dominant model							
CC	7	11.1	38	19			
CT	34	54	103	51.5			
TT	22	34.9	59	29.5	2.256	0.324	
Recessive model							
CC+CT [R]	41	65.1	141	70.5			
TT	22	34.9	59	29.5	0.660	0.417	1.28 [0.70–2.34]
Dominant model							
CC [R]	7	11.1	38	19			
CT+TT	56	88.9	162	181	2.102	0.147	1.18 [0.79–4.44]
TLR9-rs352140 SNP							
Co-dominant model							
CC	28	44.4	44	22.0			
CT	21	33.3	107	53.5			
TT	14	22.2	49	24.5	12.923	0.002*	
Dominant model							
CC+CT	49	77.8	151	75.5			
TT [R]	14	22.2	49	24.5	0.136	0.712	1.14 [0.58–2.23]
Recessive model							
CC	28	44.4	44	22.0			
CT+TT [R]	35	55.6	156	78	12.139	<0.001*	5.67 [2.91–11.06]

The chi-square test is used. *P* is set significant if ≤ 0.05, significant values are marked by *

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *LN* Lupus nephritis, *OR* Odds ratio, *CI* Confidence interval, *R* Reference genotype

In contrast, in a familial study using the transmission disequilibrium testing (TDT) analysis in a Caucasian SLE cohort of subject/parent trios (199 affected patients, 75 unaffected siblings, and 326 parents), Hawn et al. [13] reported that the TLR5/Arg392Stop variant was associated with protection from developing SLE. However, in the present study, we found no evidence to support a significant association between the TLR5-rs5744168 variant and susceptibility to or protection from SLE. This inconsistency can be related to the genetic background, methodology differences (TDT vs case-control), and/or population sampling differences between the two studies. It is possible that the effect of TLR5 is more pronounced in familial SLE than in sporadic SLE.

Interestingly, our results revealed a significant association between the TLR5-rs5744168 polymorphism and increased risk of LN. The results of the present study showed that the T allele of the TLR5-rs5744168 SNP is significantly more frequent in SLE patients with LN

Table 5 Comparison of allele distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between SLE patients with and without LN

	SLE patients without LN		SLE patients with LN		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
C allele [R]	268	97.8	119	94.4			
T allele	6	2.2	7	5.6	3.110	0.078	2.63 [0.86–7.99]
TLR9-rs187084 SNP							
C allele [R]	119	43.4	48	38.1			
T allele	155	56.6	78	61.9	1.010	0.315	1.25 [0.81–1.92]
TLR9-rs352140 SNP							
C allele	146	53.3	77	61.1			
T allele [R]	128	46.7	49	38.9	2.143	0.143	0.73 [0.47–1.12]

The chi-square test is used. *P* is set significant if ≤ 0.05

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *SLE* Systemic lupus erythematosus, *OR* Odds ratio, *CI* Confidence interval, *R* Reference allele, *LN* lupus nephritis

compared to the controls. In addition, the TT genotype of the TLR5-rs5744168 SNP was found in 3.2% of the SLE patients with LN while none of the SLE patients without LN or controls had the TT genotype.

These findings were consistent with the findings of Elloumi et al. [27] who observed a significant association between the TLR5-rs5744168 polymorphism and LN. They also reported that TT and TC genotypes of TLR-5 showed a significantly increased risk of developing renal involvement and that the TT genotype was exclusively found in LN patients. In addition, our genotypic frequencies are in accordance with those reported by Sanchez and co-workers [34], who found a significant increase frequency in the CC genotype in SLE patients without LN compared to LN patients. The relationship between the TLR5-rs5744168 polymorphism and LN was also supported by a murine genetic study, which observed that the SLE-1d locus, corresponding to the human chromosome 1q41–1q42, is associated with development of LN [35].

Another major finding of the present study is in the TLR9-rs3532140 SNP, the C allele and homozygous CC genotype in co-dominant and recessive models were significantly more prevalent in SLE patients than controls and were also significantly more prevalent in SLE patients with LN than controls. In addition, in the TLR9-rs3532140, the CC genotype in recessive models was more prevalent in the SLE patients with LN patients than those without LN. However, allelic and genotyping distribution of TLR9-rs187084 SNP showed no association with increased risk of development of SLE or LN.

The results of the present study showed no significant difference between SLE patients and controls regarding the allelic and genotypic distribution of TLR9-rs187084 polymorphism. In agreement with our findings, the results obtained by Elloumi et al. [27] indicated the absence of relationship between TLR9-rs187084 polymorphism and SLE and LN.

Similar results were also reported in studies of different Asian populations. In China, a study that included 799 healthy Chinese blood donors and 467 patients with SLE found that TLR9-rs187084 SNPs were similar in both the SLE patients and controls [22]. The results of another study from Japan that included 198 controls and 183 SLE patients showed no significant difference of the genotyping distribution of TLR9-rs187084 SNPs between the SLE patients and controls. In addition, a study from India compared the TLR9-rs187084 SNP genotyping between 62 SLE patients without nephritis and 50 LN patients and found no significant difference between the two groups. In contrast, results of a meta-analysis that pooled data from 26 studies that included 11, 984 SLE patients and 14, 572 controls indicated a significant association between the two alleles of the TLR9-rs187084 polymorphism and SLE in the overall population [36].

Regarding the TLR9 rs351240 variant, our results revealed that the C allele and the CC genotype significantly associated with increased susceptibility to develop SLE and LN. In agreement with our findings, Elloumi et al. [27] reported that the C allele and CC genotype were associated with a significantly increased risk of developing SLE with nephritis. On the contrary, the pooled data of a previous meta-analysis did not support relationship between the two alleles of the rs352140 polymorphism and the increased risk of developing SLE [36]. Consistent with the findings of the present study, the results of two previous studies supported the association between rs 351240 and increased risk for SLE, but with the T allele in the dominant model [37, 38]. These inconsistent results of the association between TLR-9

Table 6 Comparison of genotyping distribution of TLR9 gene SNP rs187084 and TLR5 gene SNP rs5744168 between SLE patients with and without LN

	SLE patients without LN		SLE patients with LN		Chi-square test		*OR [95% CI]*
	n	%	*n*	%	X^2	*P*	
TLR5-rs5744168 SNP							
Co-dominant model							
CC	131	95.6	58	92.1			
CT	6	4.4	3	4.8			
TT	0	0.0	2	3.2	4.421	0.110	
Recessive model							
CC+CT [R]	137	100.0	61	96.8			
TT	0	0	2	3.2	4.393	0.036*	11.18 [0.53–26.34]
Dominant model							
CC [R]	131	95.6	58	92.1			
CT+TT	6	4.4	5	7.9	1.050	0.305	1.88 [0.55–6.42]
TLR9-rs187084 SNP							
Co-dominant model							
CC	22	16.1	7	11.1			
CT	75	54.7	34	54.0			
TT	40	29.2	22	34.9	1.189	0.552	
Recessive model							
CC+CT [R]	97	70.8	41	65.1			
TT	40	29.2	22	34.9	0.661	0.416	1.30 [0.69–2.46]
Dominant model							
CC [R]	22	16.1	7	11.1			
CT+TT	115	83.9	56	88.9	0.852	0.356	0.64 [0.26–1.58]
TLR9-rs352140 SNP							
Co-dominant model							
CC	41	29.9	28	44.4			
CT	64	46.7	21	33.3			
TT	32	23.4	14	22.2	4.479	0.107	
Dominant model							
CC+CT	105	76.6	49	77.8			
TT [R]	32	23.4	14	22.2	0.031	0.859	0.94 [0.46–1.91]
Recessive model							
CC	41	29.9	28	44.4			
CT+TT [R]	96	70.1	35	55.6	4.023	0.045*	1.87 [1.01–3.47]

The chi-square test is used. *P* is set significant if ≤ 0.05, significant values are marked by *

TLR Toll-like receptor, *SNP* Single nucleotide polymorphism, *SLE* Systemic lupus erythematosus, *OR* Odds ratio, *CI* Confidence interval, *R* Reference genotype, *LN* lupus nephritis

gene polymorphisms and SLE may be attributed to the different genetic background among different population groups or ethnicities.

The limitations of the study and the future research

This case-control study possesses some potential limitations that worth some consideration. Firstly, all SLE patients and control volunteers were recruited from one locality in the study may not be representative of the genotype distribution of the general Egyptian population. However, the genotypic distribution of the healthy control references in this study fulfilled HWE. Secondly, due to a limited sample size, data from one case-control study may be not sufficient to fully reveal the association between the TLR polymorphisms and predisposition to SLE or LN. Therefore, the results of this study must be supported by studies with larger populations. Thirdly, it is likely that the increased risk for SLE or LN is not attributed to a single SNP of TLR gene; however, the risk must be interpreted

with consideration to other SNPs of other genetic factors, as well as various environmental factors. Finally, the accurate implication of the associations between TLR gene SNP and increased risk for SLE or LN must be confirmed by further future studies in various ethnicities.

Conclusion

Our results support the potential role of TLR5-rs5744168 SNP and TLR9-rs3532140 SNP not only in increasing the risk for development of SLE, but also in increasing the risk of LN in SLE patients among the Egyptian population.

Abbreviations

CI: Confidence interval; HWE: The Hardy-Weinberg equilibrium; LN: Lupus nephritis; OR: Odds ratio; SD: Standard deviation; SLE: Systemic lupus erythematosus; SNPs: Single nucleotide polymorphisms; TDT: The transmission disequilibrium testing; TLRs: Toll-like receptors.

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Authors' contributions

All authors contributed in the study conception and design. *OG*, *SB*, and *MA* participated in the data acquisition and analysis, statistical analysis, drafting, and revising the manuscript. *MZ*, *SE*, *ME*, and *EE* were responsible for doing the laboratory investigations and analyzing the data, statistical analysis, drafting, and revising the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The research was approved by the Institutional Review Board (IRB)/Mansoura Faculty of Medicine/Mansoura University (R.19.08.581). The written consent was obtained from all participants and approved by IRB.

Consent for publication

Not applicable in this study as there are no personal details.

Competing interests

The authors declare that they have no competing interests.

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