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RESEARCH

Toll-like receptor 10 gene polymorphism and risk of multiple sclerosis among Iraqi patients

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Abstract

Background: Toll-like receptors (TLRs) are a family of 10 pattern recognition receptors (TLR1–TLR10) involved in the regulation of inflammatory and immune responses besides their role in the pathogenesis of autoimmune diseases including multiple sclerosis (MS). TLR10 is the least studied TLR in MS, and data for single nucleotide polymorphisms (SNPs) of the *TLR10* gene are limited. Therefore, a case–control study was performed on 85 patients with relapsing–remitting MS and 86 healthy controls (HC) to explore SNPs in the promoter region of *TLR10* gene. A 927-bp region was amplified, and Sanger sequencing identified 10 SNPs with a minor allele frequency ≥ 10% (rs200395112 T/A, rs201802754 A/T, rs201228097 T/A, rs113588825 G/A, rs10004195 T/A, rs10034903 C/G, rs10012016 G/A/C, rs10012017 G/T, rs33994884 T/Deletion [Del] and rs28393318 A/G).

Results: *Del* allele and T/Del genotype of rs33994884, as well as AG genotype of rs28393318, showed significantly lower frequencies in MS patients compared to HC. Allele and genotype frequencies of the 10 SNPs showed no significant differences between MS patients classified according to the Expanded Disability Status Scale. Haplotype analysis revealed that haplotype A-T-A-G-A-G-G-T-A showed a significantly increased frequency in MS patients compared to HC (odds ratio [OR] = 9.70; 95% confidence interval [CI] = 1.28–73.31; corrected probability [pc] = 0.03), while frequency of A-T-A-G-T-C-A-T-G haplotype was significantly decreased (OR = 0.10; 95% CI = 0.01–0.85; pc = 0.05).

Conclusions: The study indicated that two SNPs may influence susceptibility to MS (rs33994884 and rs28393318), but haplotype analysis of *TLR10* gene SNPs was more informative.

Keywords: Relapsing–remitting multiple sclerosis, Toll-like receptor 10, Single nucleotide polymorphism, Expanded disability status scale, Haplotype

Background

Multiple sclerosis (MS) is the most common type of neurological disability with a global prevalence of 35.9/100,000 in 2020 [1]. In Iraq, although a lower prevalence was estimated (11.73/100,000), the incidence of MS has increased from 0.05 in 2000 to 1.5 in 2017 [2]. It is a chronic autoimmune disorder affecting the central nervous system (CNS) primarily characterized by inflammation, demyelination, gliosis, and neuronal loss [3]. Clinically, multiple sclerosis is primarily classified into four phenotypes including relapsing–remitting multiple sclerosis (RRMS), primary progressive MS, secondary progressive MS and progressive relapsing MS, with RRMS being the most common phenotype accounting for 85% of cases [4].

The underlying cause of MS and the pathogenic mechanisms involved are still not fully understood, although complex interactions between genetic and environmental factors almost certainly play an important role [5]. Viral infections, particularly Epstein–Barr virus (EBV),

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are among the important environmental risk factors that have been linked to the etiology of MS [6]. Immunological and epidemiological studies support a causal role of EBV in risk of MS and disease progression. In this context, several immune-based mechanisms have been proposed to explain the etiological role of EBV in MS including molecular mimicry or cross-reactivity between EBV epitopes and self-epitopes and bystander damage due to aberrant immune response primarily directed to EBV [7]. Among the immune components that control antiviral responses in infected cells or in effector immune cells are Toll-like receptors (TLRs), which are indicated to play an important role in mediating immune responses against various viruses including EBV [8]. Further, TLRs expressed by astrocytes and microglia are important sensors of tissue damage and infection in the CNS and thus are involved in the initiation of innate immune response and inflammation at these sites [9].

TLRs are pattern recognition receptors (PRRs) involved in recognizing pathogen-associated molecular patterns (PAMPs) in viruses and bacteria. They are essential components of the innate immune system whose activation regulates inflammatory responses and establishes antigen-specific adaptive immunity. In humans, 10 functional TLRs (TLR1–TLR10) have been described to date [10]. Dysregulated expression of TLRs has been shown to correlate with immune imbalances, suggesting an increased risk of inflammatory and autoimmune diseases, and similar evidence has been indicated in neuroimmune diseases. Besides, the potential of TLRs as therapeutic targets in these diseases has been suggested [11, 12]. In MS, studies have indicated that TLRs play a critical role in pathogenesis of disease; for instance, TLR3 and TLR4 expression was shown to be significantly up-regulated in MS lesions, and both TLRs were positively correlated with MS pathogenesis, particularly in patients with RRMS [13]. Moreover, T helper (h) 17 cells expressing TLR2, TLR4 and TLR9 were also positively correlated with neuronal dysfunction, as well as number of active brain lesions in MS patients [14]. For TLR10, the data available in MS are limited, but it has been indicated that interferon-β may regulate the expression of distinct TLRs, including TLR3, TLR7, TLR9 and possibly TLR10, in plasmablastic dendritic cells of MS patients [15, 16].

TLR10 shows a high level of expression in immune cells (eosinophils, dendritic cells, neutrophils and B cells) and non-immune cells such as trophoblasts, but functionally, it is considered an orphan receptor with poorly understood ligands and functions, which remains an area in need of elucidation [17]. However, it has been found that TLR10 may function as a broad negative regulator of TLR-mediated signaling, and thus, its role in controlling immune responses has been suggested [18]. Additional data highlighted that TLR10 can be considered as a novel regulator of innate immune responses with a role in the differentiation of primary monocytes into functional dendritic cells [19]. Recently, it has been discussed that TLR10 can suppress other TLRs by competing with other stimulatory TLRs, while its role in promoting or reducing inflammation is subject to speculation [20]. In rheumatoid arthritis, an autoimmune disorder, increased expression of TLR10, has been found in B-cell subsets and was positively correlated with disease activity [21]. Based on these findings, it is reasonable to hypothesize that TLR10 may have a role in the pathogenesis of MS.

In humans, the *TLR10* gene (ID: 81,793) is located on the short arm of chromosome 4 (4p14) and harbors large numbers of single nucleotide polymorphisms (SNPs) [20]. These SNPs have been associated with the risk of autoimmune thyroid disease, tuberculosis and infection-related gastritis, along with their role in altering the balance between pro-inflammatory and anti-inflammatory responses [22]. In MS, to the researcher's best knowledge, the *TLR10* gene polymorphism was the subject of only one study and no association with the disease was found [23]. Therefore, this study sought to give a view of the association between SNPs located in a noncoding region of *TLR10* gene (promoter region) and risk of MS, as these polymorphisms may affect the expression of *TLR10* gene and thus may influence susceptibility to MS. In addition, we examined whether these polymorphisms are associated with the degree of disability in MS patients (Extended Disability Status Scale; EDSS).

Methods

MS patients and controls

During January 2020—June 2021, a case–control study was performed on 171 subjects, including 85 patients with RRMS and 86 healthy controls (HC). Patients were recruited from the MS clinic at the General Hospital for Neurosciences in Baghdad, and the 2010 revised McDonald criteria for RRMS diagnosis were followed [24]. Physical disability was assessed using EDSS, and as previously recommended, MS patients were divided into two groups: < 3.0 (57.6%) and ≥ 3.0 (42.4%) [25]. The HC group included blood donors and health service personnel, who did not have neurological or autoimmune disorders.

Detection of TLR10 gene SNPs

Venous blood (3 mL) was collected from patients (during their visit to the clinic) and HC in an ethylene-diamine-tetra-acetic acid (EDTA) tube. DNA was isolated from EDTA blood using a gSYNC DNA extraction kit following the manufacturer's instructions (Geneaid Biotech Ltd, Taiwan).

The complete DNA sequence of the *TLR10* gene and SNP data was downloaded first (http://asia.ensembl.org), and then, forward (5′-CCTGTAGTCCCAGACATTTG-3′) and reverse (5′-GGTTGTTAACCATCCTCTTCT-3′) primers were designed to amplify a promoter region (927 bp) using Geneious software version 11.1.2 [26]. Primers were tested online using in silico PCR analysis (https://genome.ucsc.edu/cgi-bin/hgPcr), which confirmed specificity of primers and molecular size of amplified region (chr4:38,782,390–38,783,316). The target region was screened for SNPs with a minor allele frequency ≥ 10%, and in view of this, 10 SNPs were identified (rs200395112 T/A, rs201802754 A/T, rs201228097 T/A, rs113588825 G/A, rs10004195 T/A, rs10034903 C/G, rs10012016 G/A/C, rs10012017 G/T, rs33994884 T/Deletion [Del] and rs28393318 A/G).

The PCR mix included 2 µL template DNA, 1µL of each primer (10 pmol),12.5 µL GoTaq Green Master Mix (2X: Promega, USA) and 8.5 µL nuclease-free water (total volume = 25 µL). The thermal cycler (BioRad, USA) was set for the following optimized conditions: one cycle of initial denaturation (95 °C for 5 min), followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 60 s, and a final extension cycle at 72 °C for 7 min. Amplified DNA products were subjected to forward and reverse sequencing (Sanger sequencing, Macrogen Corporation, South Korea) and analyzed to identify 10 SNPs of *TLR10* gene by aligning with reference sequences (http://asia.ensembl.org). Genotypes of *TLR10* gene SNPs were determined using Geneious software version 11.1.2 [26]. A representative chromatogram of DNA sequences of three SNPs (rs201228097, rs201802754 and rs200395112) are shown in Fig. 1.

Statistical analysis

Alleles and genotypes of *TLR10* gene SNPs were presented by a number and a percentage, and significant differences were assessed using Fisher's exact test or Pearson's Chi-square test. Pearson's Chi-square goodness-of-fit test was used to test for Hardy–Weinberg equilibrium (HWE). Logistic regression analysis was applied to calculate odds ratio (OR) and 95% confidence interval (CI) after adjusting for age and gender. A probability (p) value ≤ 0.05 was considered statistically significant. Due to multiple comparisons, Bonferroni correction was applied to correct p-value (pc). The statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was employed to perform statistical analysis. Haploview software version 4.2 was used to assess linkage disequilibrium (LD) between SNPs and to estimate haplotype frequencies. Logarithm of odds (LOD) and LD coefficient (D') were used to define LD.

Results

Age and gender distribution of participants

The mean age of RRMS patients was 35.0 ± 8.8 years (age range = 18–59 years), which corresponds to the mean age of HC (mean age = 36.4 ± 9.5 years; age range = 22–50 years). In addition, males showed similar frequencies in patients and HC (45.9 and 46.5%, respectively).
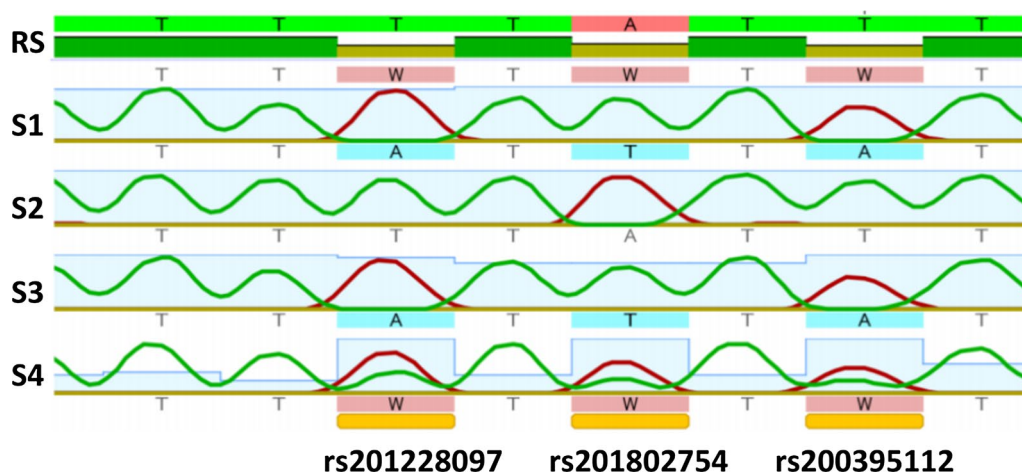
Fig. 1 DNA sequence chromatogram of three *TLR10* gene SNPs (rs201228097, rs201802754 and rs200395112) showing genotypes of four samples (S1, S2, S3 and S4). Besides, reference sequence (RS) is also given. The chromatogram was generated by Geneious software analysis (version 11.1.2)

TLR10 gene SNPs

Genotype frequencies of the 10 *TLR10* gene SNPs (rs200395112 T/A, rs201802754 A/T, rs201228097 T/A, rs113588825 G/A, rs10004195 T/A, rs10034903 C/G, rs10012016 G/A/C, rs10012017 G/T, rs33994884 T/Deletion [Del] and rs28393318 A/G) were in good agreement with HWE in HC. Comparisons between MS patients and HC revealed that only two SNPs showed significant differences (rs33994884 and rs28393318). *Del* allele (21.8 *vs.* 36.0%; OR = 0.49; 95% CI = 0.30—0.81; $p = 0.006$; $pc = 0.018$) and T/Del genotype (17.6 *vs.* 37.3%; OR = 0.31; 95% CI = 0.15—0.65; $p = 0.003$; $pc = 0.009$) of rs33994884 showed significantly lower frequencies in MS patients compared to HC. rs28393318 is the second SNP that showed a significant difference between MS patients and HC. The heterozygous genotype AG of this SNP was observed to have a lower frequency in patients compared to HC (21.2 *vs.* 45.3; OR = 0.29; 95% CI = 0.14—0.62; $p = 0.002$; $pc = 0.006$). Allele and genotype frequencies of the remaining eight SNPs showed no significant differences between MS patients and HC (Table 1). Further, allele and genotype frequencies of the 10 SNPs showed no significant differences between the two EDSS groups of MS patients (< 3.0 and ≥ 3.0) (Table 2).

LD and haplotype analysis

Haploview software was used to determine LD between 9 SNPs of *TLR10* gene (rs200395112, rs201802754, rs201228097, rs113588825, rs10004195, rs10034903, rs10012016, rs10012017 and rs28393318). SNPrs33994884 was excluded due to the presence of *Del* allele. These SNPs showed different profiles of LD; strong LD (LOD > 2 and D' = 1), weak LD (LOD > 2 and 0.21 < D' < 1) or no LD (LOD < 2 and D' < 1). For instance, rs201228097, rs201802754 and rs200395112 SNPs were in a strong LD (Figs. 1 and 2).

Haplotype analysis revealed two important findings. The haplotype A-T-A-G-A-G-G-T-A (in the order: rs200395112, rs201802754, rs201228097, rs113588825, rs10004195, rs10034903, rs10012016, rs10012017 and rs28393318) showed a significantly increased frequency in MS patients compared to HC (0.102 *vs.* 0.003; OR = 9.70; 95% CI = 1.28—73.31; $p = 0.003$; $pc = 0.03$), while frequency of A-T-A-G-T-C-A-T-G haplotype was significantly decreased (0.003 *vs.* 0.058; OR = 0.10; 95% CI = 0.01—0.85; $p = 0.005$; $pc = 0.05$) (Table 3).

Discussion

Although the etiology of MS is not well known and the pathogenesis is not fully understood, recent compelling epidemiological and molecular data have indicated the significance of the combined effects of multiple risk factors in determining an individual's susceptibility to

disease progression [3]. It is notably recognized that the initiation of MS involves a chronic dysregulation of immune homeostasis resulting from complex interactions between infectious exposures, genetic predispositions and factors associated with increased pro-inflammatory conditions, including obesity, low sun exposure/low vitamin D levels and smoking [6]. This is reinforced by epigenetic alteration studies suggesting that epigenetic alternations may be involved in the initiation and progression of MS, possibly by correlating the effects of environmental risk factors and host genetics [27]. Accordingly, genetic predisposition is the cornerstone of MS etiology, and in fact, genome-wide association studies (GWAS) revealed more than 200 loci implicated in susceptibility to disease [28]. GWAS also uncovered that up to 10.5% of the genetic variance underlying MS risk is linked to human leukocyte antigen (HLA) class II alleles among all ethnic groups studied [29]. However, studies have also revealed that non-HLA loci and their SNPs may influence susceptibility to MS, especially those involved in controlling components involved in innate and adaptive immune responses; for instance, cytokines and TLRs [12, 16, 30, 31].

Recently, *TLR* gene SNPs have been discussed as important determinants of susceptibility to several autoimmune diseases including MS, and TLR genetic variations may shed light on the role of complex interaction between immune and environmental factors in influencing the susceptibility to MS in various populations [32]. In this context, several SNPs within *TLR* genes have been linked to altered susceptibility to MS [23, 33, 34]. The less researched member of *TLR* genes is *TLR10* gene, and only one study identified five SNPs (rs4129008, rs4129009, rs11466657, rs11096955 and rs11096957) in Caucasian MS patients from Denmark but no association with the disease has been demonstrated [23]. None of these SNPs was located in the promoter region. Although promoter SNPs do not alter protein structure, they are involved in the regulation of transcription events and can alter the affinity for normal transcription factors [35]. Therefore, the current study was conducted to examine SNPs in the promoter region of *TLR10* gene. Of the 10 SNPs identified, two showed a significant association with MS. The first was rs33994884, and the *Del* allele and T/Del genotype of this SNP were associated with a reduced risk of developing MS (OR = 0.49 and 0.31, respectively). There is no direct evidence to support these findings but indicated that *Del* alleles of some SNPs within *TLR* genes may have functional effects and thus may alter susceptibility to diseases. An example is two deletion polymorphisms within *TLR2* gene. The *Del* allele of a 22-bp nucleotide deletion polymorphism in the promoter region of *TLR2* gene has been

Table 1 Logistic regression and Hardy–Weinberg analyses of *TLR10* gene SNPs in multiple sclerosis patients and healthy controls

SNP	Allele/genotype	MS[a]		HC[a]		OR	95% CI	*p*-value (*pc*)
		N	%	N	%			
rs200395112 T/A	*T*	52	31.3	20	32.3	Reference		0.874 (1.0)
	A	114	68.7	42	67.7	1.04	0.56–1.94	
	TT	11	13.3	1	3.2	Reference		
	TA	30	36.1	18	58.1	0.15	0.02–1.21	0.082 (0.246)
	AA	42	50.6	12	38.7	0.32	0.04–2.51	0.434 (1.0)
HWE-*p*-value				0.067				
rs201802754 A/T	*A*	48	28.9	20	32.3	Reference		
	T	118	71.1	42	67.7	1.17	0.63–2.19	0.629 (1.0)
	AA	9	10.8	1	3.2	Reference		
	AT	30	36.1	18	58.1	0.19	0.02–1.50	0.142 (0.426)
	TT	44	53.0	12	38.7	0.41	0.05–3.28	0.672 (1.0)
HWE-*p*-value				0.067				
rs201228097 T/A	*T*	50	30.1	20	32.3	Reference		
	A	116	69.9	42	67.7	1.10	0.59–2.06	0.75 (1.0)
	TT	9	10.8	1	3.2	Reference		
	TA	32	38.6	18	58.1	0.20	0.02–1.60	0.148 (0.444)
	AA	42	50.6	12	38.7	0.39	0.05–3.13	0.672 (1.0)
HWE-*p*-value				0.067				
rs113588825 G/A	*G*	147	88.5	75	87.2	Reference		
	A	19	11.5	11	12.8	0.88	0.40–1.94	0.838 (1.0)
	GG	67	80.7	32	74.4	Reference		
	GA	13	15.7	11	25.6	0.56	0.23–1.39	0.238 (0.714)
	AA	3	3.6	0	0.0	3.37	0.18–64.40	0.55 (1.0)
HWE-*p*-value				0.336				
rs10004195 T/A	*T*	81	48.2	49	53.3	Reference		
	A	87	51.8	43	46.7	1.22	0.74–2.03	0.517 (1.0)
	TT	24	28.6	12	26.1	Reference		
	TA	33	39.3	25	54.3	0.66	0.28–1.55	0.391 (1.0)
	AA	27	32.1	9	19.6	1.50	0.55–4.09	0.605(1.0)
HWE-*p*-value				0.534				
rs10034903 C/G	*C*	84	50.6	62	53.5	Reference		
	G	82	49.4	54	46.5	1.12	0.70–1.80	0.717 (1.0)
	CC	27	32.5	14	24.1	Reference		
	CG	30	36.1	34	58.6	0.46	0.20–1.02	0.072 (0.216)
	GG	26	31.3	10	17.2	1.35	0.52–3.51	0.626 (1.0)
HWE-*p*-value				0.175				
rs10012016 G/A/C	*G*	143	84.1	132	79.5	Reference		
	A	27	15.9	34	20.5	0.73	0.42–1.28	0.322 (1.0)
	GG	62	72.9	55	66.3	Reference		
	GA	19	22.4	22	26.5	0.77	0.38–1.56	0.475 (1.0)
	AA	4	4.7	6	7.2	0.59	0.16–2.18	0.519 (1.0)
HWE-*p*-value				0.09				
rs10012017 G/T	*G*	89	53.0	91	55.5	Reference		
	T	79	47.0	73	44.5	1.11	0.72–1.70	0.661 (1.0)
	GG	28	33.3	26	31.7	Reference		
	GT	33	39.3	39	47.6	0.79	0.39–1.58	0.590 (1.0)
	TT	23	27.4	17	20.7	1.26	0.56–2.84	0.677 (1.0)
HWE-*p*-value				0.736				

Table 1 (continued)

SNP	Allele/genotype	MS[a]		HC[a]		OR	95% CI	*p*-value (*pc*)
		N	%	N	%			
rs33994884 T/Del	*T*	133	78.2	96	64.0	Reference		
	Del	37	21.8	54	36.0	0.49	0.30–0.81	**0.006 (0.018)**
	TT	59	69.4	34	45.3	Reference		
	T/Del	15	17.6	28	37.3	0.31	0.15–0.65	**0.003 (0.009)**
	Del/Del	11	12.9	13	17.3	0.49	0.20–1.20	0.161 (0.483)
HWE-*p*-value				0.101				
rs28393318 A/G	*A*	102	60.0	80	53.3	Reference		
	G	68	40.0	70	46.7	0.76	0.49–1.19	0.258 (1.0)
	AA	42	49.4	23	30.7	Reference		
	AG	18	21.2	34	45.3	0.29	0.14–0.62	**0.002 (0.006)**
	GG	25	29.4	18	24.0	0.76	0.35–1.66	0.547 (1.0)
HWE-*p*-value				0.439				

p: Two-tailed Fisher's exact probability; pc: Bonferroni correction probability(significant *p*-value is indicated in bold)

SNP single nucleotide polymorphism, *Del* deletion, *HWE* Hardy–Weinberg equilibrium, *MS* multiple sclerosis, *HC* healthy controls, *OR* odds ratio adjusted for age and gender, *CI* confidence interval

[a] The number of patients and controls for each SNP varies due to low-quality reads of some DNA sequences

linked to increased susceptibility to breast cancer [36], while the heterozygous genotype of this allele has been associated with a protection from cerebral malaria [37]. Another *Del* allele of a polymorphism in *TLR2* gene (-196 to -174 Ins/Del; rs111200466) has been associated with increased susceptibility to aggressive periodontitis, type 2 diabetes mellitus and prostate cancer [38–40], while in primary gouty arthritis, no association was found with this allele [41]. A recent study demonstrated that the heterozygous genotype of rs111200466 showed a lower frequency in patients with schizophrenia than in HC, and thus, a protective tendency against this psychiatric disorder has been suggested, particularly in women [42]. The presented studies disclosed interesting findings, which indicated that the heterozygous genotype of the *Del* allele may be associated with a protective trend against some diseases rather than conferring susceptibility. This view is shared by the current study, and therefore, it is possible to suggest that the T/Del genotype of rs33994884 may have a protective effect against the development of MS.

The study also showed that the rs28393318 AG genotype was associated with a lower risk of developing MS (OR = 0.29). No previous study could confirm or refute these findings, but it has been demonstrated that rs28393318 was among 38 variants that showed an association with asthma and allergic diseases [43]. Another study showed that rs28393318 was associated with the presence of precancerous gastric lesions [44]. Genome-wide genetic studies also showed that rs28393318 was among 17 genetic loci that had an effect on the

abundance of transcripts of immune genes especially those related to cytokines [45]. Taken together, these data may indicate that rs28393318 can affect innate immune signaling and thus may contribute to the inflammatory response in MS.

Although single-SNP-based association studies have great potential for identifying genetic influences on diseases of complex traits, a haplotype-based analysis that incorporates LD information from several SNPs may be more powerful and informative than the traditional analysis that focuses on individual SNPs [46]. In view of this, a multi-locus haplotype analysis that included nine *TLR10* gene SNPs (in the order: rs200395112, rs201802754, rs201228097, rs113588825, rs10004195, rs10034903, rs10012016, rs10012017 and rs28393318) was performed. The analysis revealed two important findings; A-T-A-G-A-G-T-A haplotype was significantly associated with a 9.70-fold increased risk of MS, while A-T-A-G-T-C-A-T-G haplotype was associated with a reduced risk of MS. As these blocks included both risk and protective haplotypes, a critical role for the *TLR10* promoter towards MS susceptibility could be envisaged, but more studies are certainly warranted to understand this role in MS pathogenesis.

Regardless of the significance of this study being the first to explore the association between genetic variants in the *TLR10* promoter region and MS in Iraqis, some limitations should be noted. First, the sample size of patients and HC is relatively small compared to other case–control studies. Second, EBV status (prevalence and

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Table 2 Allele and genotype frequencies of *TLR10* gene SNPs stratified by Expanded Disability Status Scale groups in multiple sclerosis patients

SNP	Allele/genotype	EDSS				*p*-value
		< 3.0		≥ 3.0		
		N	%	N	%	
rs200395112 T/A	*T*	29	30.8	23	31.9	1.0
	A	65	69.2	49	68.0	
	TT	5	10.6	6	16.7	0.558
	TA	19	40.4	11	30.6	
	AA	23	48.9	19	52.8	
rs201802754 A/T	*A*	25	26.6	23	31.9	0.492
	T	69	73.4	49	68.1	
	AA	4	8.5	5	13.9	0.722
	AT	17	36.2	13	36.1	
	TT	26	55.3	18	50.0	
rs201228097 T/A	*T*	27	28.7	23	31.9	0.733
	A	67	71.3	49	68.1	
	TT	4	8.5	5	13.9	0.724
	TA	19	40.4	13	36.1	
	AA	24	51.1	18	50.0	
rs113588825 G/A	*G*	81	86.2	66	91.7	0.33
	A	13	13.8	6	8.3	
	GG	36	76.6	31	86.1	0.551
	GA	9	19.1	4	11.1	
	AA	2	4.3	1	2.8	
rs10004195 T/A	*T*	47	49.0	34	47.2	0.877
	A	49	51.0	38	52.8	
	TT	13	27.1	11	30.6	0.618
	TA	21	43.8	12	33.3	
	AA	14	29.2	13	36.1	
rs10034903 C/G	*C*	48	51.1	36	50.0	1.0
	G	46	48.9	36	50.0	
	CC	15	31.9	12	33.3	0.890
	CG	18	38.3	12	33.3	
	GG	14	29.8	12	33.3	
rs10012016 G/A/C	*G*	81	82.7	62	86.1	0.672
	A	17	17.3	10	13.9	
	GG	35	71.4	27	75.0	0.767
	GA	11	22.4	8	22.2	
	AA	3	6.1	1	2.8	
rs10012017 G/T	*G*	46	47.9	43	59.7	0.160
	T	50	52.1	29	40.3	
	GG	12	25.0	16	44.4	0.158
	GT	22	45.8	11	30.6	
	TT	14	29.2	9	25.0	
rs33994884 T/Del	*T*	72	73.5	61	84.7	0.092
	Del	26	26.5	11	15.2	
	TT	32	65.3	27	75.0	0.22
	T/Del	8	16.3	7	19.4	
	Del/Del	9	18.4	2	5.6	

Table 2 (continued)

SNP	Allele/genotype	EDSS					*p*-value
		< 3.0		≥ 3.0			
		N	%	N	%		
rs28393318 A/G	*A*	61	62.3	41	56.9		0.142
	G	37	37.7	31	43.1		
	AA	25	51.0	17	47.2		0.788
	AG	11	22.4	7	19.4		
	GG	13	26.5	12	33.3		

For simplicity, the patients were divided into two groups: < 3.0 [no to moderate disability] and ≥ 3.0 [higher disability]); *p*: Pearson Chi-square test probability

SNP single nucleotide polymorphism, *Del* deletion, *EDSS* Expanded Disability Status Scale (The EDSS scale ranges from 0 [normal neurological exam] to 10 [death due to MS] in 0.5 unit increments that represent higher levels of disability
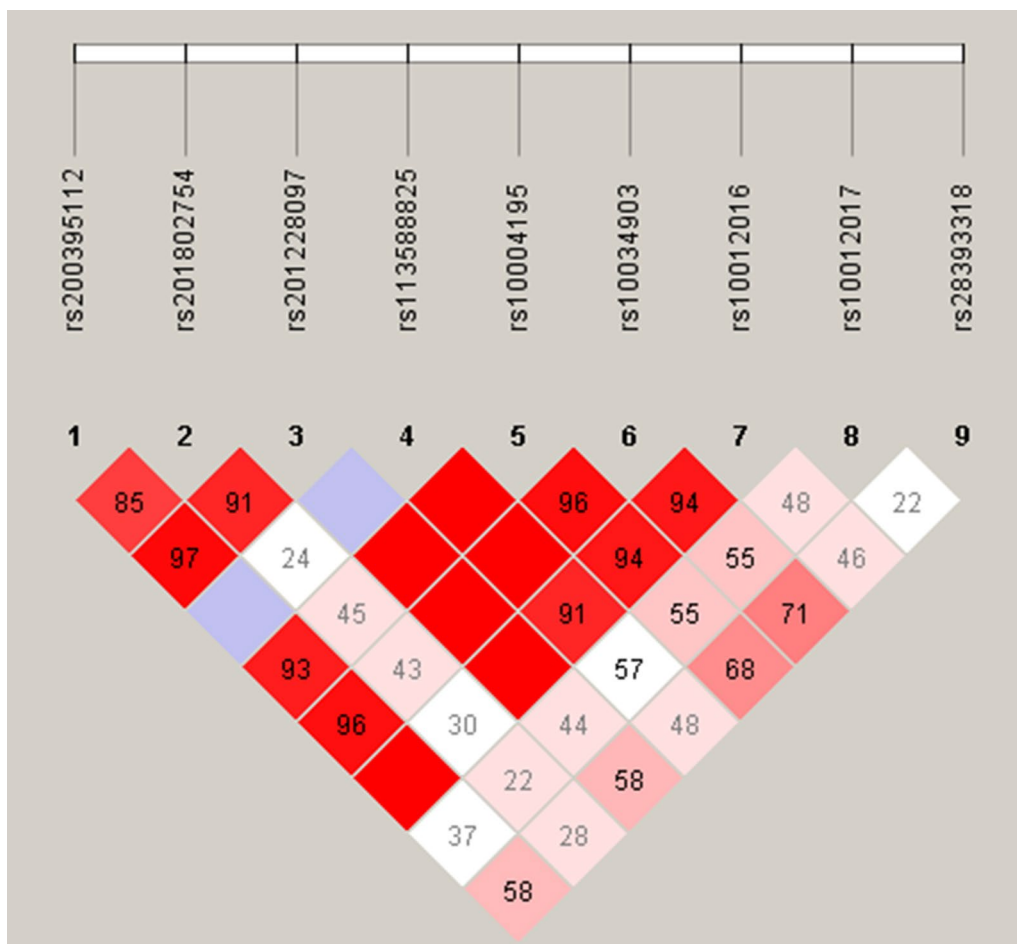
Fig. 2 Linkage disequilibrium (LD) map of nine SNPs within the *TLR10* gene. LD blocks were generated using the default algorithm in Haploview software (version 4.2). The coloring of the boxes is based on the scores of two estimates, logarithm of odds (LOD) and LD coefficient (D′). Strong LD (LOD > 2 and D′ = 1) is indicated in red. A decrease in the intensity of red indicates lower LOD and D′ values. Regions with weak LD (LOD > 2 and 0.21 < D′ < 1) are shown in blue. Regions with no LD (LOD < 2 and D′ < 1) are shown in white. The number within each box indicates the D′ statistic value multiplied by 100

Table 3 Estimated frequencies of nine-locus haplotypes of *TLR10* gene SNPs (in the order: rs200395112, rs201802754, rs201228097, rs113588825, rs10004195, rs10034903, rs10012016, rs10012017 and rs28393318) among multiple sclerosis patients and healthy controls

Haplotype	Frequency			OR	95% CI	*p*-value (*pc*)
	All	MS	HC			
A-T-A-G-A-G-G-G-A	0.230	0.241	0.210	1.20	0.64–2.24	0.637 (1.0)
T-A-T-G-T-C-G-T-G	0.154	0.132	0.198	0.62	0.31–1.24	0.175 (1.0)
A-A-A-G-A-G-G-G-A	0.109	0.091	0.144	0.61	0.27–1.37	0.231 (1.0)
A-T-A-G-A-G-G-T-A	0.069	0.102	0.003	9.70	1.28–73.31	**0.003 (0.03)**
T-A-T-G-T-C-G-G-G	0.061	0.088	0.009	8.44	1.11–64.30	**0.012** (0.12)
T-A-T-G-T-C-G-T-A	0.060	0.051	0.079	0.58	0.20–1.64	0.381 (1.0)
A-T-A-A-T-C-A-T-G	0.049	0.039	0.069	0.48	0.16–1.46	0.294 (1.0)
A-T-A-G-A-G-G-G-G	0.030	0.018	0.052	0.38	0.08–1.71	0.136 (1.0)
A-A-A-A-T-C-A-T-A	0.023	0.012	0.045	0.25	0.05–1.38	0.098 (0.98)
A-T-A-G-T-C-A-T-G	0.022	0.003	0.058	0.10	0.01–0.85	**0.005 (0.05)**

p: Pearson Chi-square test probability; *pc*: Bonferroni correction *p*-value. Significant *p*-value is indicated in bold

MS multiple sclerosis, *HC* healthy controls, *OR* odds ratio, *CI* confidence interval

viral load) was not determined and it would be of interest to correlate it with the *TLR10* polymorphism. Third, the impact of *TLR10* polymorphism on serum level and/or gene expression of TLR10 was not studied.

Conclusions

The study indicated that two SNPs in the promoter region of *TLR10* gene may influence susceptibility to MS. The T/Del genotype of rs33994884 and AG genotype of rs28393318 are suggested to have protective effects against the development of MS. Haplotype analysis of *TLR10* gene SNPs was more informative in highlighting the association with MS susceptibility.

Abbreviations

CI: Confidence interval; CNS: Central nervous system; D': Linkage disequilibrium coefficient; Del: Deletion; EBV: Epstein–Barr virus; EDSS: Expanded Disability Status Scale; LD: Linkage disequilibrium; LOD: Logarithm of odds; MS: Multiple sclerosis; OR: Odds ratio; *p*: Probability; PAMP: Pathogen-associated molecular pattern; *pc*: Corrected probability; PRR: Pattern recognition receptors; RRMS: Relapsing–remitting multiple sclerosis; SNP: Single nucleotide polymorphism; TLR: Toll-like receptor.

Declarations

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