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Methyl-CpG-Binding Protein 2 (MECP2) Polymorphism in Iranian Patients with Systemic Lupus Erythematosus

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Abstract—Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which involves many organs and presents with various symptoms. It has been shown that genetic and environmental factors play a major role in this disease and may affect the onset, activity, damage, and mortality of the disease. According to recent studies, methyl-CpG-binding protein 2 (*MECP2*) has been associated with SLE in various populations. Herein, we studied *MECP2* polymorphism in Iranian lupus patients and controls. The study included a total of 884 samples of Iranian ancestry (492 independent SLE patients and 392 unrelated healthy controls). Healthy controls were gender-, ethnic-, and age-matched with the patients. Patient and control samples were genotyped for rs1734787, rs1734791, rs1734792, and rs17435 by applying the Allelic Discrimination Real-Time PCR System. Our results showed a significant association between rs1734787 and rs1734791 SNPs and the risk of SLE in the Iranian population ($p=0.028$, $p=0.028$), but did not show any significant association with rs1734792 and rs17435 SNPs ($p=075$, $p=0.75$). The rs1734787 C and the rs1734791 T allele frequencies in the patients were significantly higher than the control group ($p=0.014$, $p=0.012$). In addition, a significant CTAT haplotype frequency was observed in cases with SLE ($p=0.012$), and a significant AAAT haplotype frequency was observed in the control group ($p=0.0003$). However, there was no significant association between genotype frequencies and SLE patients. Also, there was no significant association between these SNPs and clinical features. The result of this study suggests that polymorphism in the *MECP2* locus is associated with the susceptibility of Iranian SLE patients.

KEY WORDS: systemic lupus erythematosus (SLE); *MCEP2*; polymorphism; iranian population.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which is linked to the connective tissue. It may involve many organs of the body such as the kidneys, skin, lungs, heart, the hematopoietic system, and the brain [1, 2]. This disease mostly affects women between 20 and 40 years of age and occurs nine times more often in women than in men [3]. SLE patients produce various pathogenic autoantibodies, most of them are non-specific. Two kinds of the produced autoantibodies are almost specific; anti-dsDNA and anti-phospholipid [4]. The SLE etiology is partially known; nonetheless, it has been shown that genetic and environmental factors play an important role in this disease [5]. Several genes impact the probability of developing lupus when actuated by the environmental factors. The most important genes, which are associated with SLE, are located in the HLA region on chromosome 6 [6]. Several genes which contain the risk variant for SLE are *IRF5*, *PTPN22*, *STAT4*, *ITGAM*, *BLK* [7], *TNFSF4*, and *BANK1* [8]. Another gene, which contains the risk variant for SLE according to numerous studies, is Methyl-CpG-binding protein 2 (*MECP2*). The major role of this gene is it's binding to methylated cytosine in CpG islands and recruits histone deacetylase complexe to promoter sequences of target genes, which leads to the deacetylation of histone residues. The consequence of this process is the increment of the interaction between DNA

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and histone proteins, which induces heterochromatin formation that is inaccessible for the transcription factors and results in the suppression of gene expression [5]. Also, *MECP2* has an ability to recruit DNA methyltransferase1 (*DNMT1*), which causes inactivation of gene expression. Single nucleotide polymorphisms (SNPs) of the *MECP2* gene have been associated with SLE pathogenesis in some studies [9, 10]. A study on individuals of European descent showed an association between lupus and variants within the *MECP2* gene [11]. Another study regarding gene association in SLE concluded that the susceptibility of genes including *MECP2* was correlated with SLE in individuals of European ancestry [10]. A study of the Korean population confirmed that there were significant associations between the variants of SNPs within *MECP2* in Korean patients and systemic lupus erythematous [5]. Due to the important role of *MECP2* in this disease, our aim was to investigate whether SNPs of *MECP2* are associated with susceptibility and clinical features of SLE in Iranian patients.

MATERIALS AND METHODS

Patients and Controls

SLE patients were recruited from the outpatient rheumatology clinic of Shariati Hospital, Tehran, Iran, from February 2012 to April 2014. The study included a total of 884 samples of Iranian ancestry (492 independent SLE patients and 392 unrelated healthy controls). The patient group had a mean age of 37.5±12.2 years and included 428 females and 64 males. The mean age of the healthy controls was 38.5±13 years (324 females and 68 males) and they had neither clinical evidence nor family history of any type of autoimmune disorder. The healthy controls were gender-, ethnic-, and age-matched with the patients (Table 1). All cases fulfilled the revised criteria of the American College of Rheumatology for the classification of SLE [12]. All patients were seen by a rheumatologist for the diagnosis of SLE. Informed consent was obtained from all patients and controls. In order to exclude intra-family correlation, neither two SLE patients nor two controls from the same family were chosen. Five milliliters of blood samples were obtained from all subjects for extraction of genomic DNA. Clinical features and laboratory tests for the classification of the disease were performed in SLE patients. The correlation between various genotypes of each SNP with clinical features of the disease was evaluated. The Human Research Ethics Committee of Tehran University of Medical Sciences approved this study.

Genotyping

The genomic DNA was extracted from the whole blood using the phenol–chloroform method [13]. The extracted DNA samples were stored at −20 °C. Approximately 30 ng of the genomic DNA of each sample was used for genotyping. Amplification was performed in 10-µl reaction volumes, containing 5 µl of the TaqMan Genotyping master mix (PN, 4371355), 0.25 µl of TaqMan Genotyping assay mix (PN, 4351376), 0.25 µl of distilled water, and 4.5 µl of genomic DNA. Genotyping of *MECP2* was performed using the MGB-TaqMan Allelic Discrimination method (Applied Biosystems, Foster City, CA, USA). Patient and control samples were genotyped for rs1734787, rs1734791, rs1734792, and rs17435 by applying the StepOnePlus Real-Time PCR System (Applied Biosystems). The PCR reaction was done in 96-well plates on a programmable thermal cycler. The PCR cycle includes a denaturation phase at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and at 60 °C for 1 min.

Statistical Analysis

Genotype and allele distribution between case and control groups were evaluated by the Chi-Square test.

Table 1. Ethnic Groups

Ethnic subject	Case		Control	
	Count	Percent, %	Count	Percent, %
Fars	253	51.4	202	51.5
Turk	151	30.7	121	30.9
Kurd	45	9.2	33	8.4
Lor	31	6.3	24	6.1
Gilak	12	2.4	12	3.1
Total	492	100	392	100

Table 2. Linkage Disequilibrium Tests

D':	rs1734791	rs1734792	rs17435
rs1734787	0.986	0.183	0.173
rs1734791	–	0.188	0.179
rs1734792	–	–	0.978

According to SHEsis online analysis tool, there were about 98 % linkage between rs1734787 and rs1734791 polymorphisms and 98 % linkage between rs1734792 and rs17435 polymorphisms
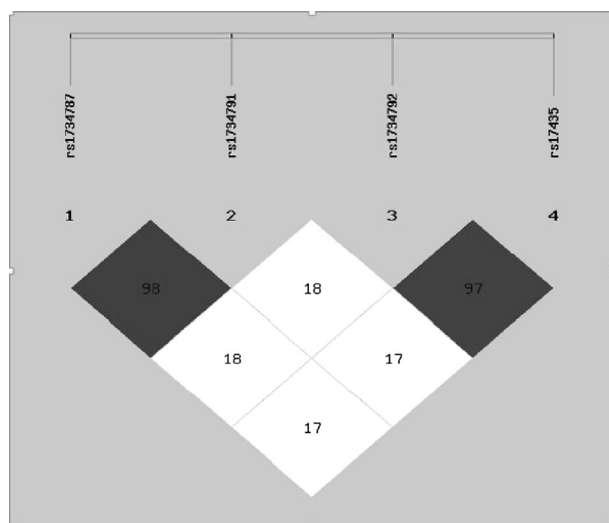
Fig. 1. Schematic diagram for Linkage Disequilibrium tests. According to SHEsis online analysis tool, there were about 98 % linkage between rs1734787 and rs1734791 polymorphisms and 98 % linkage between rs1734792 and rs17435 polymorphisms

The Pearson Chi-Square test was applied in order to determine the association between genotype and allele

frequencies, and risk of SLE. An alpha of 0.025 was regarded as the significant level. All p values were two-tailed. The odds ratio (OR) and 95 % confidence interval (CI) were also calculated. The genotype distributions of rs1734787, rs1734791, rs1734792, and rs17435 were tested for deviation from the Hardy–Weinberg equilibrium in cases and controls. The Bonferroni correction method was used to correct multiple comparisons, controlling the false discovery rate (FDR) [14]. Statistical analyses were carried out using SPSS for Windows (version 22.0, IBM SPSS Inc., USA). SHEsis online software was used for haplotype, genotype analysis, linkage disequilibrium, and also Hardy–Weinberg equilibrium [15].

RESULTS

We genotyped four SNPs (rs1734787, rs1734791, rs1734792, and rs17435) in 492 independent SLE patients and 392 unrelated healthy controls. The distribution of these genotypes did not show any significant deviation from the Hardy–Weinberg equilibrium between patients

Table 3. Allele and Genotype Distribution of MECP2 in Systemic Llupus Erythematosus (SLE) Patients and Healthy Controls

dbSNP	Alleles/genotypes	SLE (n=492) N (%)	Control (n=392) N (%)	P value	Adj. P^a	OR (95 % CI)
rs1734787	C	512 (52.0)	362 (46.2)	0.014	0.028	1.26 (1.04–1.52)
	A	472 (48.0)	422 (53.8)	0.014		0.79 (0.65–0.95)
	CC	144 (29.3)	93 (23.7)	0.065	0.26	1.33 (0.98–1.80)
	AC	224 (45.5)	176 (44.9)	0.8	0.9	1.03 (0.79–1.34)
	AA	124 (25.2)	123 (31.4)	0.042	0.084	0.74 (0.55–0.99)
HWE		0.051	0.055			
rs1734791	T	516 (52.4)	364 (46.4)	0.012	0.028	1.27 (1.05–1.53)
	A	468 (47.6)	420 (53.6)	0.012		0.79 (0.65–0.95)
	TT	144 (29.3)	92 (23.5)	0.53	0.706	1.35 (0.99–1.83)
	AT	228 (46.3)	180 (45.9)	0.90	0.9	1.02 (0.78–1.33)
	AA	120 (24.4)	120 (30.6)	0.039	0.084	0.73 (0.54–0.98)
HWE		0.115	0.128			
rs1734792	C	481 (49.0)	378 (48.2)	0.75	0.75	1.03 (0.85–1.24)
	A	501 (51.0)	406 (51.8)	0.75		0.97 (0.80–1.17)
	CC	127 (25.9)	86 (21.9)	0.175	0.35	1.24 (0.91–1.70)
	AC	227 (46.2)	206 (52.6)	0.062	0.248	0.78 (0.59–1.01)
	AA	137 (27.9)	100 (25.5)	0.42	0.51	1.13 (0.84–1.53)
HWE		0.096	0.30			
rs17435	T	528 (53.8)	431 (55.0)	0.61	0.75	0.95 (0.79–1.15)
	A	454 (46.2)	353 (45.0)	0.61		1.05 (0.87–1.27)
	TT	150 (30.6)	122 (31.1)	0.85	0.85	0.97 (0.73–1.30)
	AT	228 (46.4)	187 (47.7)	0.71	0.9	0.95 (0.73–1.24)
	AA	113 (23.0)	83 (21.2)	0.51	0.51	1.11 (0.81–1.53)
HWE		0.144	0.471			

SLE systemic lupus erythematosus, *Adj. P^a adjusted p* value for multiple testing using Bonferroni correction method, *HWE* Hardy–Weinberg equilibrium; OR, odds ratio; CI, confidence interval, Significance level<α=0.025

Table 4. Haplotype analysis (all those frequency<0.03 will be ignored in analysis.) Loci chosen for hap-analysis: rs1734787, rs1734791, rs1734792, rs17435

Haplotypes*	Case (freq)	Control (freq)	Chi^2	p Value	Odds ratio [95 % CI]
AAAT	176.15 (0.179)	192.91 (0.246)	13.081	0.000301	0.653 [0.518~0.823]
AACA	272.01 (0.277)	199.82 (0.255)	0.739	0.390119	1.099 [0.886~1.362]
CTAT	314.49 (0.320)	205.29 (0.262)	6.239	0.012516	1.307 [1.059~1.613]
CTCA	177.96 (0.181)	141.48 (0.180)	0.012	0.911924	0.986 [0.772~1.261]

*Haplotypes with frequency more than 0.03 were selected for haplotype analysis

and healthy controls. The association analysis between rs1734787 and rs1734791 SNPs and the risk of SLE in the Iranian population showed a significant association. Our data showed about 98 % linkage between rs1734787 and rs1734791 polymorphisms and 98 % linkage between rs1734792 and rs17435 polymorphisms (Table 2, Fig. 1). The rs1734787 C allele, and the rs1734791 T allele frequencies in the patients were significantly higher than the control group (Adj. P^a=0.028, OR=1.26, 95 % CI=1.04–1.52, and Adj. P^a=0.028, OR=1.27, 95 % CI=1.05–1.53 respectively). There were no significant association between the genotype frequencies and SLE (Table 3). In addition, a high CTAT haplotype frequency was observed in cases with SLE (P=0.012, OR=1.307, 95 % CI=1.059–1.613). Furthermore, a high AAAT haplotype frequency was observed in the control group (P=0.0003, OR=0.653, 95 % CI=0.518–0.823; Table 4). We next investigated whether these two SNPs (rs1734787 and rs1734791) were correlated with clinical manifestations of SLE, such as the malar rash, discoid rash, photosensitivity, aseptic necrosis, oral ulcer, arthritis, pleuritic, proteinuria, psychosis, pericarditis, vasculitis, hepatitis, leukopenia, anemia, hematuria, thrombocytopenia, anti-dsDNA, CH50 complement, and ANA (Table 5). After the Bonferroni correction, there was no significant association between the SNPs and clinical features (Data not shown). The result of this study suggests that the MCEP2 gene may play a role in the pathogenesis of Iranian SLE patients.

DISCUSSION

Systemic lupus erythematosus (SLE) is a chronic and systemic autoimmune disease that presents with various symptoms. The immune system is widely endangered in SLE patients, and malfunction of the regulatory system might lead to breakage of tolerance and cause many problems [16]. It has been shown that the

incidence and prevalence rate and also the clinical and laboratory features are quite different in various populations. Furthermore, it has been reported that genetic and environmental factors can affect the onset, activity, damage, and mortality caused by the disease [17–19]. Epidemiological data suggests that the SLE concordance rate in monozygotic twins (24–69 %) is higher than the dizygotic twins (2–5 %). The data also confirms the role of genetics in SLE [20]. Several genes that have been determined as risk factors (odds ratios generally between 1.15 and 2.0) for SLE are include; MHC region genes, IRF5, ITGAM, STAT4, BLK, BANK1, PDCD1, PTPN22, TNFSF4, TNFAIP3, SPP1, IRAK1, MECP2, TREX1, C2, C4, C1q, and some of the Fcγ receptors [21]. As mentioned earlier,

Table 5. Demographic features and manifestation of SLE patients

Clinical feature	Count (%)
Malar rash	282 (57.3)
Photosensivity	307 (62.4)
Palmar erythema	33 (6.7)
Vasculitis ulcers	40 (8.1)
Discoid rash	62 (12.6)
Serositis	95 (19.3)
Mucosal lesion	201 (40.8)
Arthritis	285 (57.9)
Aseptic necrosis	27 (5.5)
Muscle weakness	66 (13.4)
Raised muscle enzyme	55 (11.2)
Myositis in muscle biopsy	17 (3.5)
Leukopenia	201 (40.8)
Lymphopenia	168 (34.1)
Hemolytic anemia	88 (17.9)
Anemia	285 (58)
Thrombocytopenia	130 (26.4)
Neusychiatric manifestations	95 (19.3)
Renal manifestations	325 (66)
Proteinuria	318 (64.6)
Hematuria	188 (38.2)
Cellular cast	82 (16.6)

several studies have shown that the *MECP2* is a susceptibility gene for SLE patients in different populations [11, 22]. *MECP2* is an approximately small gene that is located on the X chromosome [5]. It is known the *MECP2* gene has four exons which are translated into two isoforms, i.e., E1 and E2 [23]. The E1 isoform has 498 amino acids and encoded by exons 1, 3, and 4, whereas the E2 isoform has 486 amino acids and encoded by exons 2, 3, and 4 [24, 25]. MeCP2 is a protein that binds to the methylated DNA. It also binds to the unmethylated DNA with a weaker affinity. Furthermore, it has a high affinity and specificity for methylated CpG than other methyl-DNA-binding family members [26–29]. The consequence of the binding of MeCP2 to the methylated binding domain (MBD) is the recruitment of HDAC, which induces heterochromatin formation and finally gene suppression [30]. Another controversial role of MeCP2 is the recruitment of CREB1 as a co-activator and ultimately in gene expression. In a study by Webb *et al.* in European-derived lupus patients, it was shown that the expression level of CREB1 was higher in patients with the lupus-risk *MECP2* haplotype than patients with the protective haplotypes. In the meantime, the reverse was true in the case of HDAC1. We selected the four mentioned SNPs to analyze whether these SNPs in the Iranian population are associated with SLE. In this case–control study, we showed that rs1734787 and rs1734791 polymorphisms of the *MECP2* gene were significantly associated with SLE and could be risk factors of developing SLE. The result of these two SNPs (rs1734787 and rs1734791) was congruent with the results of other studies in other countries, but it was interesting that the result of two other SNPs (rs1734792 and rs17435) was different from other studies. The different results of different studies may be explained by differences in the sample size and genetic predisposition. Our findings suggest that the C allele of rs1734787 and the T allele of rs1734791 polymorphisms may increase the susceptibility to SLE. The risk allele of the rs1734787 polymorphism in studies by Webb *et al.* in European-derived lupus patients, Kaufman *et al.* in four different ancestries, Sawalha *et al.* in a population of Korean ancestry, and also in our study was the C allele. The risk allele of the rs1734791 polymorphism in our study and the study by Kaufman *et al.* was the T allele [5, 11, 22]. On the other hand, the AAAT haplotype may protect the individuals and the CTAT haplotype may predispose the individuals to SLE. The *MECP2* gene was associated with susceptibility to SLE in the Iranian population, similar to other studies in different ethnic groups.

In conclusion, polymorphisms in *MECP2* gene might change the gene expression and because of important role of MeCP2 in regulate the other gene expression with recruit the HDAC and the CREB1, as we mentioned earlier, might alter the expression of many genes [11] which may cause unexpected immune responses and act as a starting point for inflammatory reactions in the body. Hence, the *MECP2* has a complex regulatory system that will require larger studies to assess the role of *MECP2* genotype on lupus in concert with other genetic and environmental risk factors.

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Declaration of conflict of interest. The author declares that they have no competing interests.

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