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ORIGINAL ARTICLE

Association between 318C/T polymorphism of the *CTLA-4* gene and systemic lupus erythematosus in Iranian patients

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Abstract

Background: Cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) is an important negative regulator of T-cell response. It is a functional candidate gene connected with susceptibility to systemic lupus erythematosus (SLE). We analyzed the role of −318C/T polymorphism in the promoter region of the *CTLA-4* gene in Iranian patients suffering from SLE.

Methods: A total of 180 SLE patients and 304 healthy ethnically matched controls were enrolled in the study. DNA was extracted from blood samples according to the standard procedure. Polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) was used to analyze the genotype and allele frequencies of these polymorphisms.

Results: The CC genotype was observed in 170 (94.5%) of the SLE patients, which was significantly different compared to the controls (251 [82.4%]; $P = 0.0001$, OR = 3.51 95%CI = 1.77–7.53). T allele was significantly more common in the controls (9.2%) compared to SLE patients 2.8% ($P = 0.0001$, OR = 0.26, 95%CI = 0.13–0.53). There was no significant correlation between different genotypes and age, gender or family history of SLE in the studied population.

Conclusion: It can be concluded that −318C/T polymorphism of *CTLA-4* gene might play a significant role in the development of SLE in the Iranian patients.

Key words: 318C/T, *CTLA-4*, polymorphism, promoter, systemic lupus erythematosus.

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BACKGROUND

Systemic lupus erythematosus (SLE) is an autoimmune multisystem disorder characterized by the production of multiple immunoglobulin G (IgG) autoantibodies.[1] The disease can affect multiple tissues

and organs, including kidneys, joints, skin, pleura and pericardium, diverse blood cells and the nervous system.[2] It is more frequently reported in individuals in the second, third or fourth decades of life.[3] SLE afflicts more than 1 million individuals in the US and 3.2–14.1 cases per 100 000 women of European descent.[4,5] The disease is reported in 40 per 100 000 of the Iranian population.[6] The etiology and pathogenesis of SLE still remains unknown; however genetic predisposition and environmental factors are deemed to play an important role in its pathogenesis.[7] Twin and family studies have provided evidence for the involvement of genetic factors in this disease, showing an increased concordance among monozygotic twins compared with the dizygotic ones and a high degree of familial clustering for the disease.[8] Cytotoxic lymphocyte antigen-4 (*CTLA-4*) plays an important role in regulating T cell activation, helping to limit T cell response in inflammatory conditions. It may also play a role in maintaining peripheral tolerance.[9] Similar to Graves', celiac, autoimmune thyroid disease, type I diabetes, rheumatoid arthritis and multiple sclerosis, the expression of *CTLA-4* is increased in patients with active SLE and thus the gene is believed to have a key role in the pathogenesis of the disease.[10,11] One of them is located at the −318 position in the promoter region. Using a case–control study, we determined the role of −318C/T polymorphism in SLE pathogenesis.

MATERIALS AND METHODS

Patients

One hundred and eighty SLE patients (15 male and 165 female) with a mean age of 32.99 ± 10.45 years (range 13–70 years) were enrolled in the study. Three hundred and four ethnically and age-matched healthy controls (23 male and 281 female) with no history of autoimmune diseases were recruited from the Azar 5th Teaching Hospital affiliated to Gorgan University of Medical Sciences, Gorgan, Iran. All the SLE patients fulfilled the American College of Rheumatology 1997 revised criteria for SLE.[12] The study was approved by the local ethics committee and a written informed consent was obtained from each patient.

DNA extraction and genotyping

The DNA was extracted from peripheral blood lymphocytes using a DNA extraction kit (Roche Applied Science, Penzberg, Germany) according to the standard protocol from the manufacturer. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the −318C/T polymorphism in the promoter region. PCR was carried out using the following primers: forward 5′-AAATGAATTG GACTGGATGGT-3′ and reverse 5′-TTACGAGAAAG GAAGCCGTG-3′. Amplification was carried out after initial denaturation at 94°C (2 min), followed by 30 cycles at 94°C (30 s), 60°C (30 s), 72°C (30 s) and a final extension at 72°C (7 min). The PCR products were digested using restriction enzyme *Mse*I (New England BioLabs, Hitchin, UK) at 37°C for 3 h and then were analyzed on 3% agarose gel using ethidium bromide staining. The amplified DNA consisted of 226 and 21 bp fragments (C allele) or 21, 96 and 130 bp fragments (T allele; Fig. 1).

Statistical analysis

The frequency of alleles and genotypes were assessed using direct counting. Chi-square and Fisher's exact test were used to compare the association between the alleles and genotype frequencies and SLE. *P*-values < 0.05 were considered statistically significant. The strength of the association between the incidence of SLE and the frequency of the studied alleles or genotypes of polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Statistical analysis was conducted with STATA V.8 software (StataCorp., College Station, TX, USA).

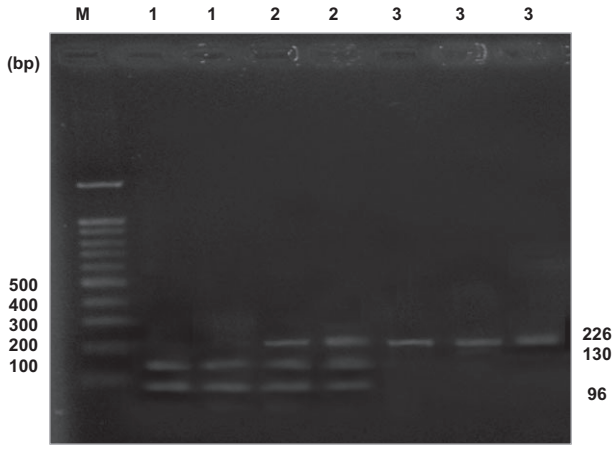

Figure 1 Genotyping of the CTLA-4 gene promoter −318 C/T polymorphism bu *Mse*I restriction fragment length polymorphism. DNA size marker (100 bp ladder). (1) TT genotype (21, 96, 130 bp); (2) CT genotype (21, 96, 130, 226 bp); (3) CC genotype (226, 21 bp). The 21 fragments are not visible on the agarose gel.

RESULTS

Approximately 86.2% of the studied patients were aged between 15 and 45 years; 91.7% of them were female and 15% had a positive family history of SLE. Table 1 shows the frequency of different genotypes and alleles for the −318C/T polymorphism in the studied group. The CC genotype was observed in 94.5% of the SLE patients and 82.4% of the controls; the difference was statistically significant (odds ration [OR] = 3.51; $P = 0.0001$). The distribution of the genotype in the control group was in Hardy–Weinberg equilibrium.

The CT genotype, on the other hand, was more frequently observed in the control group (17.1% *vs*. 5.5%, $P = 0.0001$; OR = 0.28). The T allele was also significantly more frequent among the controls (9.2% *vs*. 2.8% in SLE patients; $P = 0.0001$; OR = 0.26). Although the C allele was more common in the patients, we found no significant difference in the frequency of the C allele between the patients and the controls ($P = 0.62$). We could not find any significant correlation between the individual ages, gender, family history of SLE and different genotypes (Table 2).

DISCUSSION

Genetics is an important risk factor for SLE. This comes while many studies have reported that different environmental factors act together to place genetically predisposed individuals at a higher risk of developing SLE.[13] In the present study, we determined a strong association between the *CTLA-4* polymorphism and susceptibility to SLE in the Iranian population. Our results showed a significant association between CC genotype and SLE, adding that CT genotype and T allele are more common in the healthy population.

Contrary to our findings, Lee *et al.*[14] reported that CT genotype was more common in SLE patients, adding that CC and TT genotypes were more frequently seen in their controls. However, the difference noted between our results could be explained by race diversity between the Iranian and Korean populations. Although this study indicates a different pattern, it is the only study to our knowledge that is in line with our findings, to show a positive correlation between −318 polymorphism and SLE.[15–21]

With respect to the *CTLA-4* gene, no association was found between the presence of *CTLA-4* and SLE in individuals from the US,[15] Malaysia,[16] Korea,[17] England,[18] Japan[19] and Spain.[20] Although the results of the study conducted by Lee *et al.*[14] differed from that of Hudson *et al.*,[17] which involved the same population (Korean), these discrepancies may arise from various features, including gender, sample size and age at the onset of disease. The results indicate that *CTLA-4* gene polymorphism is linked with SLE, whereas the majority of the studies have reported a negative association between −318C/T polymorphism and SLE.[15–21]

The inconsistent results of the present study may be partially explained by the differences in the ethnicities of the studied population. SLE, as a systemic autoimmune disease, is highly heterogenenous with regard to severity, organ involvement and autoantibody profile. Autoantibodies against a variety of autoantigens are known to be associated with organ involvement associated with the disease. Therefore, we investigated clinical futures of SLE.

Other polymorphisms in *CTLA4* gene, including −1722 T/C, −1661 A/G and +49 A/G are shown to be associated with multiple sclerosis as an autoimmune condition in the Iranian population.[22] Therefore it

Table 1 Association between *CTLA-4* −318C/T allele and SLE risk factors

Risk factor	*C* (%)	*T* (%)	Total (%)	*P*-value
Age (years)				
< 15	8 (2)	0 (0)	8 (2)	0.42
15–45	300 (83.3)	10 (3)	310 (86.3)	
More than 45	42 (11.7)	0 (0)	42 (11.7)	
Gender				
Female	321 (89.2)	9 (2.5)	330 (91.7)	0.59
Male	29 (8)	1 (0.3)	30 (8.3)	
Family history of SLE				
Yes	53 (14.7)	1 (0.3)	54 (15)	0.54
No	297 (82.5)	9 (2.5)	306 (85)	

SLE, systemic lupus erythematosus.

Table 2 Genotypic distribution and allelic frequencies of *CTLA-4* −318C/T polymorphisms in the Iranian SLE patients and healthy controls

Exon 1	SLE (%) *N* = 180	Control (%) *N* = 304	*P*-value	OR (95%CI)
Genotype				
CC	170 (94.5)	249 (81.9)	0.0001*	3.75 (1.86–7.57)
Allele				
C	350 (97.2)	552 (90.8)	0.0001	0.26 (0.13–0.53)
T	10 (2.8)	56 (9.2)		

*CC versus TT + CT.

could be hypothesized that the association of −318 CC genotype with SLE found in this study might be secondary to the disequilibrium with these single nucleotide polymorphisms (SNPs). Therefore, it will be interesting to examine the association between these SNPs individually and as haplotypes in our patients in the future.

Our study did not report any link between age, gender and having a positive family history of SLE and −318C/T genotypes. This comes while previous studies had reported *CTLA-4* −318C/T polymorphisms in SLE patients.[15–21] This is the first report of association between −318 CT polymorphism and clinical features of SLE and the first to show an association between −318CT and SLE among our population.

Studying the expression of CTLA4 in tissue and blood samples obtained from subjects with different genotypes can shed light on the mechanism of CTLA4 involvement in vulnerability to developing SLE. Previous studies have shown the functional effect of −318C/T polymorphism on the promoter activity of CTLA4[23] with the T allele corresponding to a significantly higher promoter activity compared to the C allele. Other studies have also demonstrated increased expression of CTLA-4 after stimulating the cell surface and at the messenger RNA level in non-stimulated cells in individuals carrying the T allele of this polymorphism.[24]

In conclusion, the −318C/T polymorphism appears to play a significant role in the development of SLE in the Iranian population. Therefore, further studies on populations, especially from other Middle Eastern countries, are needed to confirm our results.

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