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Original Article

CTLA-4 polymorphism in Iranian patients with systemic lupus erythematosus

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Abstract. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is an important negative regulator of T-cell responses. CTLA-4 polymorphisms have been confirmed to be associated with several autoimmune diseases such as systemic lupus erythematosus (SLE). We analyzed the role of +49AG polymorphism in exon1 of the CTLA-4 gene in Iranian patients suffering from SLE. A cohort of 180 SLE patients and 304 ethnically and age matched healthy controls were studied. Polymerase chain reaction restriction fragments length polymorphism (PCR-RFLP) was used to analyze the genotype and allele frequencies of these polymorphisms. We found that the AA genotype was significantly higher in SLE patients (67.2% vs. 41.1%, p=0.0001). The AG genotype frequency, on the other hand, was more frequently reported in the controls (49.7% vs. 27.8%, p=0.0001). The GG genotype was also more common in the control group than SLE patients but the difference was not significant (p=0.06). The frequency of G allele was significantly higher in SLE patients: 34% versus 18.9% than in control (p=0.0001). There was no significant correlation between the risk of developing SLE and the individual's age, parental consanguinity, and family history of SLE. We didn't observe any significant association between genotype and the clinical features of SLE. We conclude that the +49AG polymorphism of CTLA-4 gene appear to play a significant role in the development SLE in the Iranian patients, but not to be associated with clinical features of SLE.

Keywords: Systemic lupus erythematosus, CTLA-4, exon1, 49AG polymorphism

Introduction

Systemic lupus erythematosus (SLE) is a complex inflammatory disease characterized by autoantibody production [1]. The disease is more common in women but found in different racial and ethnic groups. It is more frequently reported in individuals in the second, third or fourth decades of life [2]. SLE affects more than 1 million individuals in the US and 3.2 to 14.1 cases per100000 in women of European descent [3, 4]. The disease is reported in is 40 per 100000 of the Iranian people [5]. The etiology of the disease is unknown but is thought to be caused by both genetic and environmental factors [6]. The expression of CTLA-4 is increased in patients with active SLE [7]. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is an important negative regulator of T-cell responses, and its dysregulation has the potential to affect the pathogenesis of SLE by altered activation of T cells to self-antigens [1]. Inappropriate T-cell dependent expansion of autoreactive B cells is considered to play a role in the production of pathogenic autoantibodies [8] in multiple organs, including kidneys, heart, lung, joints and immune system [9]. The *CTLA-4* gene is located within the risk region on chromosome 2q33 and several polymorphisms have been reported in this gene [10].

CTLA-4 polymorphisms have been confirmed to be associated with several autoimmune disorders such as, Graves' disease, type I diabetes, celiac disease, autoimmune thyroid disease, rheumatoid arthritis and multiple sclerosis and SLE [11]. One of these is located in exon 1 at position +49(A/G). Using a case-control study design, we have determined the role of +49AG polymorphism in SLE pathogenesis.

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Materials and Methods

Patients

In this study, 180 SLE patients (15 males and 165 females) with a mean age of 32.99 ±10.45 years (ranging from 13 to 70 years) were enrolled. In addition, 304 ethnically and age matched healthy controls (23 males and 281 women) with no history of any 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 & Genotyping

The DNA of from the patients and the controls was extracted from their peripheral blood with a DNA extraction kit (Roche Applied Science) according to the standard protocol from the manufacturer. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the +49AG polymorphism in exon1. +49AG polymorphism was genotyped as following using a single primer set: forward 5′-GCTCTACTTCCTGAAGACCT-3′and reverse 5′-AGTCTCACTCACCTTTGCAG-3′. Amplification was carried out after initial denaturation at 94°C (2 min), followed by thirty cycles at 94°C (30 s), 60°C (30 s), 72°C (1 min), and a final extension at 72°C (2 min). The PCR products were digested using restriction enzyme BbvI (New England BioLabs) at 65◦C for 3 h and then were analyzed on 3% agarose gel using ethidium bromide staining. The amplified DNA for was 162 bp fragment (A allele) or two fragments of 91 and 71 bp (G allele).

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 distributions and association of alleles and genotypes in the patients and the controls. A p-value <0.05 was considered as statistically significant. The strength of the association between different groups and alleles or genotypes of polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Statistical analysis was conducted with STATA (v8) software.

Linkage disequilibrium and haplotype analysis

Estimated haplotype frequencies and testing for linkage disequilibrium between pairs of polymorphisms in the cases and controls were calculated using the EHPLUS program, which provides log likelihood, chi-square and the number of degrees of freedom. To test for heterogeneity in haplotype frequencies between the cases and controls, the likelihood ratio test was used.

Results

Blood samples from 180 SLE patients and 304 controls were genotyped for the +49 AG in exon 1 region of the CTLA-4 gene. 37.2 percent of patients had consanguineous parents. Positive family history of SLE was reported in 15 percent of the patients. There was no significant correlation between the risk of developing SLE and the individual's age, parental consanguinity, and family history of SLE (Table1).

The Genotype and allele frequencies of the +49AG polymorphism are seen in Table 2. The frequency of AA genotype was significantly higher in SLE patients (67.2% vs. 41.1%, p= 0.0001; OR=2.93). The AG genotype frequency, on the other hand, was more frequently reported in the controls (49.7% vs. 27.8%, p=0.0001; OR=0.39). GG genotype was also more common in the control group than SLE patients but the difference was not statistically significant (p= 0.06; OR=0.51). The frequency of the G allele significantly increased in SLE patients: 34% versus 18.9% in control (p=0.0001; OR=0.34). However, the A allele frequency increased in patients but we observed no significant difference in the frequency of the A allele between patients and control (p=0.06). Table 3 shows the relationship between 49AG genotypes and clinical features. Although, all manifestations were more frequently reported in individuals with AA genotype, however, there was no association between CTLA-4 genotypes and clinical features of SLE (Table 3).

TABLE 1
ASSOCIATION BETWEEN CTLS-4 +49 GENOTYPE AND SLE RISK FACTORS

Risk factor	AA (%)	AG (%)	GG (%)	Total (%)	P value
Age					
<15	3 (1.6)	1 (0.5)	0	4 (2.2)	0.31
15-45	101 (56.1)	47 (26.1)	7 (3.9)	155 (86.2)	
>145	17 (9.5)	2 (1.1)	2 (1.1)	21 (11.6)	
Consanguineous parents					
Yes	50 (27.8)	15 (8.5)	2 (1.1)	67 (37.2)	0.24
No	71 (39.5)	35 (19.4)	71 (3.9)	113 (62.8)	
Family history					
Yes	21 (11.7)	6 (3.3)	0	27 (15)	0.29
No	100 (55.5)	44 (24.5)	9 (5)	153 (85)	

TABLE 2
GENOTYPIC DISTRIBUTION AND ALLELIC FREQUENCIES OF CTLA-4 +49AG POLYMORPHYSMS IN THE IRANIAN SLE PATIENTS AND HEALTHY CONTROLS

Exon 1	SLE n=180 (%)	Control n=304 (%)	P value	OR (95% CI)
Genotype				
AA	1 21 (67.2)	125 (41.1)	0.0001	2.93 (1.99-4.32)
AG	50 (27.8)	151 (49.7)	0.0001	0.39 (0.26-0.57)
GG	9 (5)	28 (9.2)	0.06	0.51 (0.23-1.12
Allele				
A	292 (81.1)	401 (66)	0.06	1.92 (0.88-4.18)
G	68 (18.9)	207 (34)	0.0001	0.34 (0.23-0.5)

Discussion

Although the definite etiopathogenesis of SLE remains unclear, evidence indicates that *CTLA-4* polymorphisms play an important role in susceptibility to SLE. Some studies have reported a significant correlation between CTLA-4 polymorphisms and SLE (13-16). On the other hand, several studies have failed to show any association

TABLE 3
ASSOCIATION BETWEEN CTLA-4 +49 GENOTYPE AND CLINICAL FEATURES OF SLE

Feature	AA (%)	AG (%)	GG (%)	Total (%)	P value
Chest pain	1 (0.5)	0	0	1 (0.5)	0.78
Oral ulcer	8 (4.5)	71 (3.8)	0	15 (8.3)	0.18
Malar rash	28 (15.5)	15 (8.3)	2 (1.1)	45 (25)	0.62
Pleurisy	5 (2.8)	4 (2.2)	0	9 (5)	0.44
Headache	13 (7.2)	5 (2.8)	2 (1.1)	20 (11.1)	0.54
Visual disturbance	8 (4.4)	1 (0.5)	0	9 (5)	0.35
Seizure	1 (0.5)	1 (0.5)	0	2 (1.1)	0.76
Renal Involvement	3 (1.6)	3 (1.6)	0	6 (3.3)	0.43
Alopecia	4 (2.2)	0	0	4 (2.2)	0.36
Myositis	21 (11.6)	8 (4.5)	0	30 (16.7)	0.87
Arthritis	43 (23.9)	13 (7.2)	2 (1.1)	58 (32.2)	0.38

between SLE and CTLA-4 polymorphisms (16-20). In this study, a strong association was observed between CTLA-4 polymorphism and SLE in the Iranian population. In line with our results, a significant correlation has been reported between 49AG polymorphism and SLE among Asian and Caucasian population (14-15, 21-22). On the other hand, several studies reported no association between this polymorphism and SLE in the different racial groups (17-21, 24-25). Possible reason behind these controversial results may arise from many aspects such as different ethnic populations, gender, sample size and age at the onset of disease. Our results indicated an association between the AA genotype and susceptibility to SLE. Corroborating with our results, Ulker et al reported that AA genotype is found more frequently among SLE patients (15). This comes while the contrary was reported for the Korean patients (14). Moreover, the AG genotype and G allele were more common in control group. This comes while previous studies had reported controversial results (14, 20-22, 26). We also investigated some of SLE risk factors such as age, having consanguineous parents and a positive family history of SLE. Having consanguineous parents was more common in patients with AA genotype. The majority of the patients with the vary genotypes (56.1%) were aged between 15 and 45 years old; the correlation however was not significant. Our study also failed to show any correlation between CTLA-4 exon 1 position 49 polymorphism and clinical features. The clinical features though were more likely to be reported in patients with AA genotype. Corroborating with our results, Ulker et al reported that patients with AA genotype were more likely to manifest SLE symptoms (15). In line with our result, the correlation was not statistically significant. Several studies have reported CTLA-4 exon 1 position + 49 gene polymorphisms in SLE patients but we could not find any study to report association between 49AG polymorphism, risk factors and clinical features of SLE patients. We found age, having consanguineous parents and a positive family history of SLE are not associated with none of the genotypes. Our study is the first study to report association between +49AG and SLE among the Iranians. Hence, further studies on other populations especially in the Middle Eastern nation are needed in this regard.

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Conflict of Interest

The authors declare no conflicts of interest.

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