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G-protein coupled-receptor 65 5´UTR gene polymorphism in the pathogenesis of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease with unknown etiology. G-protein-coupled receptor 65 (GPR65) candidates as an SLE-locus for functioning in T cell receptor-mediated self-reactive T cell death in the thymus. This is also involved in anti-inflammatory actions and apoptosis as remarkable features of autoimmune diseases. This study investigated the relationship between the rs10139328 polymorphism at the 5´UTR of a GPR65 gene and SLE. This case-control study consisted of 102 SLE patients (98 females, 4 males) and 118 age- and gender-matched healthy controls (113 females, 5 males). Genotyping of the rs10139328 polymorphism was determined using an amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Data was analyzed using SPSS software. The Pearson chi-square was the test of choice for assessing the association between the rs10139328 polymorphism and SLE. The probable influences of sunlight and family history on SLE were evaluated by performing logistic regression. Except for one heterozygote subject among the control group, the study population was homozygote for the selected polymorphism. No statistical difference was seen in genotype distribution between the cases and the controls (P> 0.05). Statistical analysis revealed that sun exposure directly increased SLE susceptibility (P < 0.001). Having a family history of SLE increased the risk of disease occurrence by more than two times (OR = 2.38, 95% CI: 1.28 - 4.41, P= 0.006). The results of the current study do not support the importance of the studied polymorphism in a GPR65 gene in the pathogenesis of SLE among southwestern Iranian patients.

Keywords: systemic lupus erythematosus, G-protein-coupled receptor 65, polymorphism.

Introduction

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune inflammatory disorder with worldwide distribution [1]. It affects both men and women, although it appears that women of childbearing age are more susceptible to this disorder [2]. SLE is characterized by various tissue injuries, e.g., skin, lung, kidney, heart, and CNS, due to the deposition of immune complexes [3]. Hyperactivation of self-reactive B cells and dysfunction of T lymphocytes are the most probable causes of insufficiency in immune complex clearance [4-6].

T cell death-associated gene 8 (TDAG8 or GPR65, gene ID: 8477), which belongs to the G-protein-coupled receptor (GPR) superfamily, is known as a novel regulator of T cell function, T cell and B cell behavior, and autoimmune inflammation [7, 8]. This G-protein-coupled receptor actively participates in the apoptosis pathway by activating the caspase-9, -8, -3, which is a common phenomenon in autoimmune diseases including systemic lupus erythematosus (SLE) [9]. T cell receptor (TCR) is a member of a complex of proteins acting in the negative selection of self-reactive immature T cells in thymus. GPR65 is exclusively expressed in the spleen, thymus, lymph nodes, and lymphocytes and upregulated during TCR-mediated cell death [10]. Mandel et al. supposed that the down-regulation of this gene may lead to the prolonged survival of self-reactive T lymphocytes, a characteristic of autoimmune diseases [11].

The functional role of the GPR65 protein in the regulation of pro-inflammatory cytokines, production of antibodies by B cells, and modulation of the function of T cells as the main deregulated mechanisms in the pathogenesis of SLE persuaded the authors to investigate the relationship between the probable functional polymorphism rs10139328 (C/T) in the 5′UTR element of the GPR65 gene and the risk of SLE among patients from southwestern Iranian population.

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Materials and Methods

Study population

A total of 102 SLE patients (mean age 32.36±8.56 years) who were treated at the Rheumatology Clinic of Khatam-al-Anbia Hospital, Bandar Abbas, Iran were enrolled in this case-control study. One hundred eighteen healthy volunteers (mean age 32.55±9.46 years) from the same region were selected as a control group. Subjects were matched for gender and age (±5). All participants of the control group stated that they had no history of rheumatoid arthritis, SLE, or other autoimmune diseases. Pregnant women were excluded from the study (in both case and control groups). Diagnoses were performed according to the American College of Rheumatology classification criteria (ACR). All clinical procedures were approved by the local Ethics Committee of Islamic Azad University, Arsanjan Branch, and all participants completed informed consent forms prior to their inclusion in the study.

To investigate the role of sunlight in the pathogenesis of SLE, the study subjects were divided into two groups according to their workplace: Outdoors (farmers, drivers, policemen, etc.) and Indoors (housewives, teachers, etc.).

Genotyping

Five ml of blood was collected in EDTA tubes from cases and controls and stored at -20 °C until DNA extraction. DNA was extracted from whole blood samples using the standard "salting out" method [12], and the rs10139328 single nucleotide polymorphism (SNP) of the candidate gene was genotyped using the touchdown amplification refractory mutation system-polymerase chain reaction (touchdown ARMS-PCR) technique. In this method, two PCR reactions were set for each individual; each reaction contained one allele specific (in this case the C allele or T allele) primer and two outer primers (non-allele specific). Primers were designed using Primer 1 software [13]. The detailed information of the primers are shown in Table 1. PCR was performed using a commercially available PCR premix (YTA, Iran), and the manufacturer's instructions were followed. An optimized protocol which uses three different annealing temperatures was performed as follows: initial denaturation step of 95 °C for 5 min, 10 cycles of 94 °C for 40 s, 65 °C for 40 s, and 72 °C for 40 s, 15 cycles of 94 °C for 40 s, 55 °C for 40 s, and 72 °C for 40 s, 10 cycles of 94 °C for 40 s, 52 °C for 40 s, and 72 °C for 40 s, followed by a final extension cycle at 72 °C for 3 min, and then cooled to 4 °C. The amplified products were separated by electrophoresis on a 2% agarose gel containing 0.5 mg/ml DNA safe stain dye.

Statistical analysis

Statistical analysis of the data was performed using SPSS version 19.0 software. Continuous variables were reported as mean±SD, and the categorical variables were presented using frequency counts. Deviation from Hardy-Weinberg equilibrium was evaluated by performing the chi-square test. Binary logistic regression was used to assess the influence of some risk factors (family history of SLE and sun exposure) on the risk of SLE disease using a 95% confidence interval (95%CI) and odds ratio (OR). The associations between genotype and allele frequencies were investigated by computing the Pearson chi-square test. $P<0.05$ was considered statistically significant.

Results

Baseline characteristics of the study samples are described in Table 2. Most patients (95.8%) and controls (96.1%) were female. Table 3 shows the genotype and allele distributions of the rs10139328 polymorphism in SLE patients and healthy controls. The control ($\chi^2 = 0.012$, df = 1, $p \geq 0.05$) population was in Hardy-Weinberg equilibrium for the genotype frequencies of the studied SNP. The Pearson chi-square test showed that there was no significant association between the rs10139328 polymorphism and SLE (Table 3). Except for one control subject who carried the mutant allele T in a heterozygous manner, the other subjects were homozygous for wild-type allele C (Table 3). About 39.2% and 11.9% of patients and controls worked outdoors, respectively. Exposure to sunlight significantly differed between cases and controls (OR= 4.79, 95% CI: 2.41 - 9.50, $P< 0.001$). The frequency of a positive family history of SLE was significantly higher in cases (35.5%) compared to controls (18.6%) and was statistically significant between patients and controls (OR = 2.38, 95% CI: 1.28 - 4.41, $P= 0.006$) (Table 4).

Discussion

SLE is a complex disorder involving the interaction of several genetic loci and environmental factors. Among these, the role of sunlight on the risk and severity of the disease has been confirmed by many investigators [14-16]. UV light exacerbates the manifestation of SLE. The present findings suggested that subjects with outdoor jobs were at higher risk of developing SLE. It may be suggested that UV light induces apoptosis,

which promotes higher release of self-antigen and subsequently more production of auto-antibodies against them [17].

GPR65, a member of the G-protein-coupled receptor family, is important in the regulation of T cell and B cell behavior in the immune system [8]. The gene for GPR65 is located on the long arm of chromosome 14 (14q31-q32.1) with several single nucleotide polymorphisms (SNPs) distributed over the entire gene sequence [18]. Evidence indicated that GPR65 may have a role in activation-induced differentiation or T cell death [19]. In human SLE patients, peripheral blood mononuclear cells (PBMCs) expressed lower levels of GPR65 compared with those cells from multiple sclerosis (MS) patients [11]. A recent study on a mouse model of MS indicated that GPR65 deficiency exacerbating the disease [20]. Therefore, the present study hypothesized that GPR65 may be a protective factor against autoimmune diseases, and GPR65 polymorphisms may be closely associated with autoimmune diseases.

The role of GPR65 in autoimmune diseases has been investigated in numerous studies. Franke et al. reported the association of GPR65 with Crohn's disease susceptibility [21]. In addition, Ke revealed the association of GPR65 rs8005161 polymorphism with type 2 diabetes (T2D) [22]. Furthermore, disturbance in the concentration of cytokines (including IL-6 and IL-10) is an obvious event in the SLE patients [23-25]. GPR65 is known as an upstream negative regulatory component of the mentioned cytokines [8]. Therefore, GPR65 seems to be one of the best candidate genes in the pathogenesis of SLE.

Table 1. SNP in GPR65 gene, its location, and the primer sequences

Gene	polymorphism	primer sequence 5′ to 3′
GPR65	rs10139328 *C/T* 5′UTR	Forward outer: CGCACACCCTTCTCATTG Reverse outer: CAACCCCCTTTTTCTATATCTATG Reverse inner (*T*): TTTTTTAATCTTATACTTACCCCTA Reverse inner (*C*): TTTTTTAATCTTATACTTACCCCTG

Table 2. Baseline characteristics of the study samples

Characteristics	Controls	Cases	P value
Total	118	102	-
Mean age (year±SD)	32.55±9.46	32.36±8.56	0.87
Age range	13-59	13-59	-
Sex ratio (M/F)	5/113	4/98	

Table 3. Genotype and allele frequencies of the rs10139328 polymorphism among SLE patients and healthy controls

rs10139328	Controls n=118 (%)	Cases n=102 (%)	OR (95%CI)	P*
Genotypes				
CC	117 (99.2)	102 (100)		
CT	1 (0.8)	0 (0.0)	1.01 (0.99- 1.03)	0.35
TT	0 (0.0)	0 (0.0)		
Allele				
C	235 (99.2)	204 (100)	1.00 (0.02- 50.39)	0.5
T	1 (0.8)	0 (0.0)		

*Pearson Chi-square test

Table 4. Association between family history of SLE, work place, and SLE risk

Risk factor	Controls n=118	Cases n=102	OR	95% CI	*P**
Family history					
Yes	22 (18.6)	36 (35.3)	2.38	1.28-4.41	0.006
No	96 (81.4)	66 (64.7)	1	reference	-
Work place					
Outdoor	14 (11.9)	40 (39.2)	4.79	2.41-9.50	<0.001
Indoor	104 (88.1)	62 (60.8)	1	reference	-

OR: Odd Ratio, CI: Confidence interval; *$P \leq 0.05$=significant

The present study investigated polymorphism rs10139328 at 5′UTR of the GPR65. Statistical analysis revealed that in both patient and control groups, the polymorphic-type homozygote TT frequency was zero. Wild-type CC genotype was also distributed evenly in both patient and control groups; thus, no statistically significant difference in the distribution frequencies of the CC genotype between the two groups was detected. In addition, the distribution frequency of the C allele was not significantly different between the two groups. This finding supported no association between the rs10139328 polymorphism and SLE in the southwestern Iranian population. Based on the results of previous studies, GPR65 was hypothesized to be a candidate gene involved in the pathogenesis of autoimmune diseases; however, to date, their exact association remains unclear. Thus, the present study investigated the correlation between GPR65 and SLE, which is a common autoimmune disease. In contrast to the authors' hypothesis, no association between the selected polymorphism in the 5′UTR region of GPR65 and susceptibility to SLE was found among patients suffering from SLE in southwestern Iran.

Calling attention to the limitations of the study may be beneficial for the further molecular investigation of SLE. The main limitation of this study is its small sample size, especially when the study samples were stratified into indoor/outdoor or positive/negative family history groups. Another limitation is the way of considering sun exposure as a dichotomous variable (indoor vs. outdoor), not a continuous variable. It is recommended that future studies investigate the association in larger populations and participants from different geographical regions to confirm these findings.

Conflicts of interest

The author declares no conflicts of interest.

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