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ORIGINAL ARTICLE

Deoxyribonuclease I gene polymorphism and susceptibility to systemic lupus erythematosus

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Abstract The DNASE1 gene is regarded as one of the susceptible genes for systemic lupus erythematosus (SLE). Recent studies have detected the presence of a variable number of tandem repeat (VNTR) polymorphisms at intron 4 in this gene. The current study aimed to investigate the influence of current polymorphism on SLE susceptibility in a sample of the Iranian population. The study included 163 patients and 180 unrelated healthy controls. The VNTR polymorphisms in the DNASE1 gene were determined by polymerase chain reaction (PCR). The genotypic frequency investigation indicated that 3/6 genotype frequency in patients affected with SLE was more than healthy controls (P=0.004). Moreover, 3/4 and 4/6 genotype frequencies in healthy cohort were further in comparison with patient cohort (P=0.0001). Findings of the present study manifested that 3/6 genotype in patients affected with SLE was significantly more than healthy controls, thus it can be regarded as a risk factor, while 3/4 and 4/6 genotypes were significantly higher in healthy controls which can be considered as a protective factor.

Keywords DNASE1 · Polymorphism · Systemic lupus erythematosus

SEL	systemic lupus erythematosus
DNASE1	deoxyribonuclease I
VNTR	variable number tandem repeat

Introduction

Systemic Lupus Erythematosus (SLE) is a prototypical human autoimmune connective tissue disorder which is triggered in predisposed individuals by unknown environmental and genetic components [1, 2]. Although SLE is seen throughout the world, it is more prevalent in some countries and also in some ethnics within a country [3–6]. Nucleosomes are the key autoantigens in SLE patients and immune complexes comprising nucleosomes are the chief reason of tissue injury [7]. Defects in clearance of probable autoantigens including DNA–protein complexes during apoptosis can lead to the failure of self-tolerance in this disease [8, 9]. In this regard, Deoxyribonuclease1 (DNASE1) as one of the susceptible genes has been implicated in a number of immune disorders and it is a remarkable candidate gene for SLE [9]. The DNASE1 is a candidate endonuclease enzyme involved in nucleosome degradation in apoptosis pathway, thus suppresses anti-DNA autoimmunity. However, its function has been demonstrated to be diminished in individuals with SLE [10]. Besides, DNASE1-deficient mice expanded typical symptoms of SLE [11]. The DNASE1 gene has situated on chromosome 16p13.3 and has nine exons [12, 13]. Several studies declared that genetic variations in DNASE1 gene seem

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to have a correlation with SLE [13–15]. Recent researches have detected the presence of a novel 56-bp variable number tandem repeat (VNTR) polymorphism in intron 4 of DNASE1 during sequencing which was designated as HumDN1 [16]. Thus, perhaps defects in DNASE1 protein, which is originated from polymorphisms, cause expansion persistence of cellular debris such as extracellular DNA and ultimately gets involved in the pathogenesis of SLE [17]. By virtue of these evidences, we aimed to highlight the association between DNASE1 gene VNTR polymorphism and susceptibility to SLE in the South East Iranian population.

Material and methods

Patients and sample collection

The individuals recruited in the present study were a total of 163 patients (13 males and 150 females) with an average age of 32.6±8.6 years confirmed with SLE fulfilling the American College of Rheumatology (ACR) criteria [18]. All patients were referred to rheumatology clinics of Imam Ali University Hospital of Zahedan since 2011 to 2013. The control group consisted of 180 healthy individuals (166 females and 14 males) with a mean age of 32.1±11.7 years and unrelated to SLE patients. Healthy subjects were recruited according to the negative antinuclear antibody (ANA) test and the absence of systemic diseases. The ethics committee of Zahedan University of Medical Sciences approved the project and informed consent was taken from all individuals.

Genomic DNA extraction and genotyping

DNA was extracted from peripheral blood leukocytes by salting out method [19]. The VNTR polymorphisms of DNASE1 were genotyped by polymerase chain reaction (PCR) with 5′-*GGACCTTTTGTTTCTTCAA*-3′ forward primer and 5′-*ACCGCAGACACCTGGTCA*-3′ reverse primer. amplification was performed with an initial denaturation step at 95 °C for 6 min, followed by 30 cycles of 30 s at 95 °C, 30 s at 60 °C, 72 °C for 1 min with a final step at 72 °C for 7 min. PCR products were verified on a 2.5 % agarose gel containing 0.5 μg/ml ethidium bromide and visualized by UV radiation.

Statistical analysis

Statistical analysis was performed using SPSS version 18 software. The Hardy–Weinberg equilibrium was estimated separately for case and control groups. Differences in genotypic and allelic distribution between patients and controls were examined by chi-square ($\chi 2$) and independent sample t tests. In addition, the odds ratio (OR) and 95 % confidence intervals (CI) were also calculated. A P value of less than 0.05 was considered statistically significant.

Results

In the present study, we analyzed the VNTR polymorphism in DNASE1 gene on 163 SLE patients and 180 healthy control individuals. Demographic data of SLE patients and control group are shown in Table 1. Dermomucus manifestations developed in 85 % of SLE patients (54 % with malar rash). Arthritis was found in 84 % of patients, whereas neuropsychiatric manifestations were observed in 17 % of patients. Lupus nephritis was developed with raised serum creatinine in 27 % of patients. Among 6 probable alleles for DNASE1 gene, we merely observed 4 alleles. Allelic and genotypic frequency distributions of the DNASE1 gene VNTR polymorphisms are shown in Table 2. We observed that 3/4 and 4/6 genotypes were more prevalent in the control group, and according to the statistically significant P values (P=0.0001), these genotypes are regarded as potent protective factors against SLE disease. Moreover, 3/6 genotype was frequent in individual patients, and by P value of less than 0.05, it was considered as a risk factor (P=0.004). The frequency of allele 5 in SLE patients is significantly more than healthy controls and suggested that individuals with this allele may be at risk to develop SLE (OR=1.715 [95%CI, 1.131to 2.604] P=0.007).

Discussion

Approximately all autoimmune diseases have genetic background, and SLE as one autoimmune disorder also shows a strong genetic predisposition [20–23]. DNASE1could be one key nuclease for the elimination of DNA from nuclear antigens at sites of high cell turnover and therefore may impede systemic lupus erythematosus (SLE) [24].

In this study, we analyzed the association of VNTR polymorphisms in the DNASE1 gene and susceptibility to SLE in Zahedan, Sistan and Baluchestan province population. Our investigation on VNTR polymorphisms of DNASE1 gene showed that, the 3/6 genotype frequency was significantly higher in SLE patients than in healthy controls which implied the possible role of this genotype in SLE susceptibility (P=0.004). Furthermore, the 3/4 and 4/6 genotype frequencies were remarkably high in the control group which indicated the protective role of these genotypes against the disease (P=0.0001). AlFadhli et al. reported that individuals with 3/4, 5/5, and also 3/5 genotypes showed an increase in DNASE1 expression. In addition, the SLE patients with frequent 4/4 genotype presented a reduction in the enzyme expression [25]. Many studies reported that reduction in the

Table 1 Demographic and clinical characteristics of SLE and healthy cohorts

Parameter	SLE patients n=163	Controls n=180	P value
Age, years, median (IQR)	33	30	0.28
Sex, *n* (male/female)	13/150	14/166	0.6
Race, *n* (%)			
Persian	82 (50)	86 (48)	0.36
Balouch	81 (50)	94 (52)	
Joint symptoms, *n* (%)	141 (87)	–	
Renal diseases, *n* (%)	36 (22)	–	
Dermomucus	135 (83)	–	
Disorder, *n* (%)			
Malar rash	87 (53)	–	
Neurological	23 (14)	–	
Disorder, *n* (%)			
Hematological	98 (60)	–	
Disorder, *n* (%)			
Oral ulcer, *n* (%)	45 (28)	–	
ANA	134 (82)	–	
Anti-dsDNA antibodies, n (%)	122 (75)	–	

serum level of DNASE1 activity is an ordinary manifestation in SLE individuals [7, 26, 27]. According to these studies, our result shows a higher frequency of 3/4, 3/5 genotypes in the healthy group and 4/4 genotype in the patient group; however, we did not observe statistically significant P value in the 4/4 and 3/5 genotypes. In contrast, AlFadhli et al. showed DNASE1 activities decrease when the expression of DNASE1 increases. Also, some studies suggested the lower DNASE1 expression is linked with reduced enzyme activities [28]. Moreover, alternative study showed that the copy number did not associate with serum DNASE1 activity in SLE patients [17]. Besides, we saw the higher frequency of allele 5 in patients than controls which revealed this allele as a risk factor in SLE patients (P=0.007; OR=1.715 (1.131–2.604)). Consistent to our findings, AlFadhli et al. found more distribution of allele 5 in the patient group but with statistically significant P value [16]. In a similar study which was carried out by AlFadhli et al., 4/5 genotype was considerably high in patients affected with SLE which is in agreement with our results. Alfadhli et al. reported that 3/5 genotype is more in patients but our

Table 2 Genotypic and allelic frequencies of DNASE1 polymorphisms in SLE patients and controls

Genotypes	SLE patients Number=163(%)	Healthy controls Number=180(%)	P value	Odds ratio
3/3	15 (9.2)	9 (5)		Ref=1
3/4	2 (1.2)	50 (27.8)	*0.0001*	0.024 (0.004–0.123)
3/5	33 (20.2)	48 (26.7)	0.050	0.412 (0.161–1.05)
3/6	17 (10.4)	0	*0.004*	–
4/4	38(23.3)	25 (13.9)	0.527	1.096 (0.416–2.88)
4/5	55 (33.7)	27 (15)	0.427	1.222 (0.474–3.144)
4/6	0	21 (11.6)	*0.0001*	–
5/6	3 (2)	0	0.279	–
Alleles				
3	82 (25.2)	116 (32.2)		Ref=1
4	133 (40.8)	148 (41)	0.117	0.787 (0.545–1.136)
5	91 (27.9)	75 (20.8)	*0.007*	1.715 (1.131–2.604)
6	20 (6.1)	21 (6)	0.243	0.742 (0.378–1.457)

study is in controversy to them. However, Fujihara et al. reported that the allele frequencies of the DNASE1 gene fluctuated among five populations [29]. The investigation of Fujihara et al. suggested that 3/3 genotype may reduce the activity of DNASE1 enzyme [30] as we observed more frequency of this genotype in the patient group. Likewise, Yasuda et al. [31] conducted a study on Japanese and German populations and observed that frequencies of allele 2 and 6 are very rare in these populations, which was similar to our findings. According to these findings, the impact of variants in DNASE1 gene causes failure in DNASE1 activity and leads to SLE susceptibility. However, further association studies with large sample size and different ethnicities are needed to confirm our findings. Moreover, the serum level and activity of this enzyme along with this polymorphism should be investigated in various populations. In short, since SLE is a complicated disease, and one enzyme could not be the only responsible protection against autoimmunity and also reduction in the enzyme activity is not merely regulated by VNTR polymorphisms, further researches, therefore, are necessary to warrant if other genes are involved in the inadequate clearance of DNA in SLE patients.

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Compliance with ethical standards

Disclosures None

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