

# Xrcc1 polymorphism and systemic lupus erythematosus



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XRCC1 Arg399Gln and Arg194Trp Gene Polymorphisms and the Risk of Systemic Lupus Erythematosus in Iranian population (a pilot study)

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*Background & objectives* : Systemic Lupus Erythematosus (SLE) is an autoimmune multisystem disease that both genetic and environmental factors are effective in predisposing to SLE. DNA repair enzymes ceaselessly checked the chromosomes to correct damaged nucleotide produced by methylation, oxidation or oxidative damage. The human XRCC1 gene is important for DNA repair. XRCC1 is located on chromosome 19q13. 2-13. 3 with 33 kb in length and 17 exons. In this study, we examined two polymorphisms in XRCC1 gene (Arg399Gln and Arg194Trp).

*Methods*: 163 SLE patients and 180 healthy controls were genotyped for the XRCC1 Arg399Gln and Arg194Trp polymorphisms by PCR-restriction fragment length polymorphism method.

*Results*: The frequencies of AA and AG genotypes of the XRCC1 Arg399Gln polymorphism were significantly lower in SLE patients than controls. No significant association observed between XRCC1 Arg194Trp polymorphism and increased risk of SLE in studied population. Furthermore, the haplotype analysis revealed that the rs25484A - rs1799782T are a risk factor for SLE.

*Interpretation & conclusions* : These findings suggest that XRCC1 Arg399Gln polymorphism may contribute to SLE pathogenesis but XRCC1 Arg194Trp polymorphism may not result in SLE.

Key Words: Systemic Lupus Erythematosus , XRCC1

## Introduction

Systemic lupus erythematosus (SLE) is a multisystem disorder and human autoimmune disease, characterized principally by increasing of B-cells activity and auto-antibodies production against self-antigens (1, 2). SLE is ten folds more common in women (3). The pathogenesis of SLE remains elusive, although the etiology of the disease is supposed to involve genetic, hormonal and environmental factors (4). Diverse auto-antigens are considered as targets for auto-antibodies in SLE (5). Antigenicity may be increased by reactive oxygen species (ROS) and drugs, therefore changes DNA conformation, which results in DNA base damage and breaks (6). DNA repair enzymes ceaselessly checked the chromosomes to correct damaged nucleotide produced by methylation, oxidation or oxidative damage(7). Base excision repair (BER) and nucleotide excision repair (NER) are two pathways that repair most of the DNA damages including ROS(8). The X-ray repair cross-complementation group1 (XRCC1) is one of the most important protein that has the role in the BER pathway(9). XRCC1, a DNA repair protein , which is involved in single-strand breaks and BER pathway , suggested to be responsible for the effective repair of DNA damage caused by active oxygen, ionization, and alkylating agents.(10) The human XRCC1 gene is 33 kb in length, and located on chromosome 19q13. 2-13. 3 , that has 17 exons.(11).

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There are more than 300 validated single nucleotide polymorphisms (SNPs) in the XRCC1 gene reported in the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP>), three of which are common (12) and lead to amino acid replacement in XRCC1 at codon 194 (exon 6, base C to T, amino acid Arg to Trp, dbSNP no. rs1799782), codon 280 (exon 9, base G to A, amino acid Arg to His, dbSNP no. rs25489) and codon 399 (exon 10, base G to A, amino acid Arg to Gln, dbSNP no. rs25487). Although the functional effects of these polymorphisms in XRCC1 have not been understood, it is suggested that amino acid changes at preserved regions may alter its function (13). This change in protein biochemistry leads to the hypothesis that variant alleles may reduce kinetics repair, thereby result in SLE susceptibility.(14)

The aim of this study was to investigate the possible effects of XRCC1 polymorphisms on SLE risk in an Iranian population.

## 2. Materials and methods

### Patients and sample collection

In this case- control study, we evaluated 163 patients affected with SLE who referred to rheumatology clinics in Zahedan city, since 2011 to 2013. They have been diagnosed with systemic lupus erythematosus according to ACR 1998 criteria (American Rheumatology Association). The control group consisted of 180 individuals. They matched from the viewpoint of age, sex and ethnic. Volunteers have negative ANA test and no systemic diseases and also no family relation with lupus patients. All participants provided written informed consent according to the Declaration of Helsinki.

## Genomic DNA extraction and genotyping

Blood samples were collected in 2 ml Na-EDTA tubes from patients and healthy controls and genomic DNA was extracted by using salting out method. The quality and quantity of extracted DNA determined by spectrophotometer apparatus. Genotypes were detected using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and tetra-ARMS-PCR techniques. The PCR primer sequences and the condition of each primer is listed in Table 1

Table 1. The PCR primer sequences

Polymorphisms	Primer sequences(RFLP)	T <sub>m</sub> (C <sup>°</sup> )
rs1799782	F: 5'GCCAGGGCCCCTCCTTCAA- 3'	60
	R: 5'TACCCTCAGACCCACGAGT- 3'	
rs25487	F: 5'- GGACTGTCACCGCATGCGTCGG- 3'	60
	R: 5'-GGCTGGGACCACC TGTGTT- 3	

Primer sequences (tetra-ARMS)

rs1799782

outer

F: 5'-CGTCCCAGGT

R: 5

CACTCCTATCTATGGGACACAG-

3

inner

F: 5-

CGGGGGCTCTCTTC

R: 5-

CACCTGGGGATGTCTTGTTGATA

CA-3

F: 5-

rs25487

outer

ACCAGCTGTGCCTT

3

R: 5-

CTGGAGTACCCCAGCCCCTGCC-

3

F: 5-GTCGGCGGCTGCCCTCACA-

inner

3

R: 5-

TGGCGTGTGAGGCCTTACCACC-

3

Each PCR products was digested according to its protocol; 10 U/ $\mu$ l of Msp I restriction enzyme (Fermentas, Lithuania) for 12 h at 37°C. Digested samples were separated by electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining.

### Statistical analysis

Statistical analysis was performed with SPSS-V- 18. Student t test was used to compare quantitative variables and  $\chi^2$  test was used to compare non-quantitative variables. Logistic regression analysis was used to assess the independent effect of each risk polymorphism on PE. Linkage disequilibrium (LD) was analyzed using Cube X software (15). Values of  $p < 0.05$  were considered statistically significant.

### Result

Demographic data of SLE patients and control group are shown in table 2. There were no significant differences in genders and ethnics between SLE patients and controls. Moreover, there was no considerable difference between the mean age between control group ( $32.1 \pm 11.7$ ) and SLE patients ( $32.6 \pm 8.6$ ,  $p = 0.4$ ).

Table 2. Demographic characteristics of SLE patients and controls

Parameter	SLE N= 163	Controls N= 180	$p$ - Value	$\chi^2$
Age (yr)	$32.6 \pm 8.6$	$32.1 \pm 11.7$	0.68	0.04

	6	7		
Sex				
(male/female)	13/150	14/166	0.6	0.04
Race N (%)				
Persian	82 (50)	86 (48)	0.36	0.27
Balouch	81 (50)	94 (52)		

According to our findings dermomyx 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.

The allele and genotype frequencies of patients with SLE and controls are shown in Table 3. All loci were in Hardy-Weinberg equilibrium. The rs1799782 and rs25484 did not have significant LD ( $D' = 0.708$ ,  $r^2 = 0.162$  and  $P < 0.05$ ) as determined by pair-wise LD estimation.

The genotype and allele frequencies of XRCC1 Arg194Trp (rs1799782) polymorphism was not significantly different between SLE patients and control group. However, the genotypic and allelic frequencies of XRCC1 Arg399Gln (rs25484) polymorphism was statistically different between two groups. The risk of SLE was lower in individuals with AG and AA genotype in compare to those with GG genotype. (OR, 0.43 [95% CI, 0.26 to 0.71];  $P =$



0.001 and 0.53[95% CI, 0.027 to 1.03). In addition, the frequency of G allele was significantly higher in SLE patients  $p=0.011$ .

Table 3. Genotypes and alleles frequency of XRCC1 polymorphisms in SLE patients and controls

genotype	case N= 163(%)	control N= 180(%)	p value	OR
Arg399Gln				
GG	64 (39.3%)	41 (22.8%)		
AG	75 (46%)	110 (61.2%)	0.001	0.436(0.267-0.712)
AA	24 (14.7%)	29 (16%)	0.044	0.530(0.271-1.034)
allele				
A	123 (37.7%)	168 (46.7%)		
G	203 (62.2%)	192 (53.3%)	0.011	1.445(1.064-1.96)

## Arg194Trp

CC	106 (65%)	112 (62.2%)		
CT	52 (32%)	65 (36.1%)	0.269	0.845(0.538-1.328)
TT	5 (3%)	3 (1.7%)	0.341	1.76(0.41-7.575)

## allele

C	264 (81%)	289 (80.3%)		
T	62 (19%)	71 (19.7%)	0.446	0.939(0.654-1.396)

Four haplotypes of the XRCC1 consist of two-alleles of each polymorphism site is shown in table 4 · we found a rise in AT haplotype frequency in patients affected with SLE that can be regarded as risk factor for this disease.

Table 4. observed haplotype of rs1799782 and rs25484

haplotype

	case	contr ol	p value	OR
rs25484 - rs1799782				
AC	0. 578	0.544	0.109	1.28 (0.946 - 1.732)
GC	0. 231	0.263	0.089	0.74(0.523 - 1.047)
GT	0. 145	0.16	0.255	0.792 (0.53 - 1.184)
AT	0. 044	0.031	0.011	4.548(1.272-16.263)

## Discussion

Systemic lupus erythematosus (SLE) is a systemic inflammatory and autoimmune disorder of connective tissues with unknown etiology. Several factors, including endocrine and metabolic, environmental and genetic factors are important in this disease (16, 17). DNA damage can be emanate from environmental factors like mutagenic elements or internal factors including reactive oxygen species resulting from physiological process (18). So far, extensive studies on the role of oxidative stress on the immune system and its role in causing various diseases and its role in changing the structure of DNA was carried out(19). Several studies have shown that breaks in DNA mixed with nuclear proteins are strong immunogens for <https://assignbuster.com/xrcc1-polymorphism-and-systemic-lupus-erythematosus/>

provoking auto-reactive antibodies (Abs) in SLE patients. DNA repair efficiency in cells is a determining factor for preventing the development of SLE (20, 21). XRCC1's function as a remarkable protein in the activity of BER factors, contribute to assess its influences on the two most prevalent polymorphisms (22). . In this study, we examined two polymorphisms in this gene. Our findings show that AA and AG genotypes are as protective factors and GG genotype as a risk factor for this disease. But the genotypic and allelic frequencies of Arg194Trp polymorphism were not significantly different between SLE patients and control group. There are several reports showing the correlation between XRCC1 genes and autoimmune diseases like rheumatoid arthritis (23). However, data on allelic variation at the XRCC1 gene in patients with SLE are limited. Benke et al . reported that DNA repair is diminished in patients with SLE (24) In 2009 Lin et al. reported that AA genotypes of Arg399Gln gene polymorphisms were significantly less frequent in SLE patients than controls in Taiwanese Han Chinese. These findings are consistent with our findings but we observed AG genotype frequency in healthy controls further than SLE patients that is opposite of Lin et al. findings (19). Bassi and Warcho, i reported that AA and AG genotypes of XRCC1 Arg399Gln polymorphisms were highly frequent in SLE patients than controls in Polish Population (25, 26). These results are completely inconsistent with our findings, because we observed high frequency of the AG and AA genotype in control group.

With respect to another variant Arg149Trp, our study failed to show any significant differences in allelic and genotypic distribution of this polymorphism among the groups. In accordance with our results, Lin et al

(19) found no association between the XRCC1 Arg194Trp and susceptibility to SLE. Also, Koyama et al. did not find any association between XRCC1 Arg194Gln and Arg399Gln polymorphisms and rheumatoid arthritis in Japanese population(27).

In light of these findings, we suggest that there is an association between the XRCC1 Arg399Gln and vulnerability to SLE in the studied population. Current Consistent with the fact that XRCC1 has key role in the BER system thus a change in the amino acid alter the structure of this protein and may be change its function.

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#### Disclosure

The authors declare that they have no conflicts of interest.

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