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# Association of HLA-DRB1 Alleles with Juvenile-onset Systemic Lupus Erythematosus (SLE) in Iranian Children

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## Abstract

### *Introduction*

Systemic Lupus Erythematosus (SLE) is a complex autoimmune and inflammatory disease. Many studies show HLA alleles can be associated with SLE. The aim of this study was to determine the association of HLA-DRB1 alleles with juvenile- onset in Iranian children.

### *Materials and Methods*

At a case – control study, 31 children with systemic lupus erythematosus (case group) who referred to Mofid Children's Hospital, Shahid Behehsti University of Medical Sciences, Tehran, and 56 healthy children (control group) were participant. Genomic DNA was extracted and HLA typing was performed by Polymerase Chain Reaction (PCR) with Sequence - Specific Primers (SSP) technique.

### *Results*

HLA- DRB1*01, HLA- DRB1*04, HLA- DRB1*11 and HLA- DRB1*13 were detected to as most frequent alleles associated with SLE in Iranian children. The frequency of HLA DRB1*08 was not significantly different in both groups (P>0.05). HLA- DRB1*07 had a higher rate of repetition in the control group than patients with SLE.

### *Conclusion*

There was a significant difference in the frequency of some alleles between patients and controls group, which could be related to susceptibility to SLE. These differences between frequencies of some alleles in both groups may help to determine the onset of lupus in children.

***Key Words***: Autoimmune, HLA- DRB1, PCR-SSP, Systemic lupus erythematosus.

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## Introduction

Systemic Lupus Erythematosus (SLE) is a complex and systemic autoimmune disease. It is characterized by diverse clinical symptoms, revealing widespread immune - mediated damage (1, 2). The common clinical features diagnosed in patients with SLE comprise of skin and joint diseases, hematological abnormalities, renal disease and neuropsychiatric complications (3-5).

Although the etiology of SLE is still unknown, genetic factors are likely to be important in susceptibility to SLE and influence presentation of disease heterogeneity and production of autoantibody in affected subjects (6-8). Some studies show that Human Leukocyte Antigen (HLA) alleles are associated with SLE. The association of HLA- DRB1*07 and HLA- DRB1*13 with lupus was analyzed in a few studies, such as that of Wilson et al. on African Americans and Barron et al. in Denmark (6, 9). Liphaus et al. also showed that HLADRB1* 01, *04, *08, *11 has a relation to SLE (10). The present study has been done on Iranian children with SLE to determine the association of HLA- DRB1 alleles in Iranian population.

## Materials and Methods

### *Patients and Controls*

We studied 31 patients (4 boys and 27 girls) with SLE who attended Outpatient Clinic of Mofid Children's Hospital during the period from April 2011 to September 2012. Patient's mean age was 11.35±3.197 years (range from 6 to 16 years).

The control group consisted of 56 healthy individuals (24 female and 32 male) who did not have any history of immune system disorders or other diseases with known genetic or hereditary predisposition. The study was approved by the local Ethics Committee of Genetic Department, Shahid Behehsti University (M.Sc. thesis, code: D/200/1951, 2012/02/09), and written informed consent was obtained from all participants or their caretakers. Diagnosis of SLE was carried out according to the revised criteria of American College of Rheumatology for juvenile SLE (3).

### *DNA Extraction and HLA-typing*

Genomic DNA was isolated from anticoagulated whole blood from each patient according to the protocol recommended by Saremi et al.(11). The study also used DNA extraction kit according to the manufacturers' recommendation (Qiagen, Hilden, Germany) for some samples.

The HLA- DRB1 alleles were identified by a polymerase chain reaction based on sequence- specific primers (PCR-SSP) technique. The method was in accordance with the procedure developed by Olerup and Zetterquist (12). In this procedure, six alleles of HLA-DRB1 were detected using specific primers in nine PCR reactions. Polymerase chain reaction was performed for 35 cycles under the following condition: 94°C for 1 min, 60°C for 1 min and 72°C for 2 min, and materials: 100 ng DNA, 10 mMTris HCl, pH 8.4, 50 mMKCl, 2 mM MgCl2, 0.001% gelatin, 0.2 mMdNTP, 10 pmol of each primer, 2 U Taq DNA polymerase and H2O up to 55 μL.

The study used specific primers amplifying a limited region of beta- actin gene, present in all samples as a positive control. The PCR products were identified by 2.5% agarose gel electrophoresis in 1X TBE buffer and the presence of specific bands was analyzed under UV light and documented by gel documentation system. Some of samples for confirming specific bands were sequenced. Using the procedure six allele HLA DRB1*01,*04,*07,*08,*11 and*13 could be detected.

### *Statistical analysis*

To compare the HLA allele frequencies of SLE patients with those of control, chi-squared test was used. Each allele frequency in SLE patients was compared with the same allele in the controls; we considered p- value of 0.05 or less as significant. The Odds Ratio(OR) was calculated with 2×2 contingency tables by SPSS software, version 19 and strength of association between HLA - DRB1 alleles and SLE was analyzed (13).

## Results

Table.1 shows the distribution of HLA-DRB1 alleles in SLE patients and controls. There were 4 boys and 27 girls with an age range o f 6 – 16 years and mean disease duration of 2.6 ±0.968 years. According to the data of Table.1, HLA-DRB1*11 was the most frequent allele in both patient and control groups [54.8% vs 33.9%, P=0.002, OR =2.48, 95% Confidence Interval (95% CI) = 1.39 – 4.40].

Table.1 demonstrates that the most frequent alleles in patient in comparison with control group were HLA-DRB1*01 (29% vs. 8.9%, OR =4.13, 95% CI =1.83–9.28), HLA-DRB1*04 (32.2% vs. 10.7%, OR=3.807, 95%, CI=1.79–8.09), HLA-DRB1*13 (48.3% vs. 12.5%, OR =6.17, 95% CI = 3.06–12.47). HLA-DRB1*11 and HLA-DRB1*07 was repeated with a greater frequency in controls in comparison with patients. No significant difference was identified in the frequency of HLA- DRB1*08 between patients and controls (9.6% vs. 7.4%).

**Table 1**: Frequency of HLA-DRB1 alleles in Iranian patients with SLE and healthy controls

| Alleles | Control (n=56) | | Patients (n=31) | | P-value | OR (95% CI) |
|---|---|---|---|---|---|---|
| | n | Frequency (%) | n | Frequency (%) | | |
| DRB1*01 | 5 | 8.9 | 9 | 29 | 0.000 | 4.13(1.83 – 9.28) |
| DRB1*04 | 6 | 10.7 | 10 | 32.2 | 0.000 | 3.807(1.79 – 8.09) |
| DRB1*07 | 7 | 12.5 | 1 | 3.2 | 0.009 | 0.207(.057 – .75) |
| DRB1*08 | 4 | 7.14 | 3 | 9.6 | 0.447 | 1.47(.53 – 94.07) |
| DRB1*11 | 19 | 33.9 | 17 | 54.8 | 0.002 | 2.48(1.39 – 4.40) |
| DRB1*13 | 7 | 12.5 | 15 | 48.3 | 0.000 | 6.17( 3.06 – 12.47) |

## Discussion

Systemic Lupus Erythematosus is a multifactorial disease, and there is strong evidence that different genetic factors have an important role in the susceptibility to develop SLE among populations from around the world (13-16).

Analysis of susceptible genes has a significant role to characterize the pathway and etiology of development in SLE, and the results may lead to improved diagnostic, prognostic tools and more specific therapies for SLE. Several studies have demonstrated that SLE is associated with certain HLA alleles in various populations (17-19). Nevertheless, there is some controversy surrounding these findings (20).

Based on our knowledge, present study is the first one in Iranian population, and it was conducted to determine the associations of HLA DRB1 alleles and SLE disease. Our findings showed significant differences between patients

and control group for HLA-DRB1*01, *04, *11 and *13.

Ramal et al. also showed HLA DRB1*13 are associated with this disease in population of Spain (2). In one study done in Japan population results showed frequency of DRB1*01 allele was significantly increased in male patients with SLE, and they found the relation of skin ulcers in SLE patients with HLA-DRB1*04. In contrast with our finding that showed HLA-DRB1*08 (9.6% vs. 7.14%) are not significantly different between patients and controls, their results demonstrates that HLA-DRB1*08 significantly increased in patients over 50 years of age (21). Huang et al. also reported significant difference in the frequency of HLA-DRB1* 08 between patients and controls in the Taiwanese population (22). Hussain et al. identified that HLA-DRB1*01 and *011 are associate with this disease in patient from Lahore-Pakistan, and our study confirms these results .They also found that HLA-DRB1*04, *07 and*08 have a protective role against SLE (23). On the contrary, in our study only HLA DRB1*07 allele has protective effect.

Among 31 patients with Juvenile SLE, one patient with familial history of SLE was identified with HLA-DRB1*07. Although HLA-DRB1*07 were more frequent in the control group compared with patient group, it is possible that it has a role in the familial transmission of SLE in Iranian population. In black South Africans with lupus erythematosus, Rudwaleit et al. also demonstrated that HLA-DRB1*07 was more common in controls in comparison with patients(24).

The frequency of HLA-DRB1*11 in our normal population was higher than *01,*04,*08 and *13, and this result accompanies the results of different studies done in Iranian population (25-28). Table. 2, shows frequencies of HLA-DRB1 alleles in normal Iranian population. In all studies mentioned in the Table, the frequency of this allele is higher than other alleles. The Frequency of HLA-DRB1*08 in our control group is less than HLA DRB1*01, *4, *7, *11 and *13 alleles. Amirzargar et al. and Yari et al. in their studies also reported that *08 allele was less frequent among these alleles in their normal group (26, 27). However, Ghaderi et al. showed that HLA DRB1*01 had less frequency in comparison with these alleles(28).

This study certainly has its own limitations. More patients and control provide more detailed and accurate information. Moreover, the studying of other loci in the HLA complex can help elucidate the molecular mechanisms of HLA association with SLE.

**Table 2**: Frequencies of HLA-DRB1 alleles in normal Iranian population by different studies and its comparison to the present study

| References / HLA | Ghaderi 2001, n=36(%) | Amirzargar 2001, n=100(%) | Yari 2007, n=46(%) | Farivar 2011, n=45(%) | Present study, n=56(%) |
|---|---|---|---|---|---|
| DRB1*01 | 2.8 | 5.5 | 5.5 | 8.9 | 8.9 |
| DRB1*04 | 9.7 | 10.5 | 10 | 11.1 | 10.7 |
| DRB1*07 | 11.1 | 6.5 | 8.3 | 11.1 | 12.5 |
| DRB1*08 | 4.2 | 1.5 | 2 | 6.7 | 7.14 |
| DRB1*11 | 29.2 | 25 | 20 | 33.3 | 33.9 |
| DRB1*13 | 5.6 | 8.5 | 11.4 | 13.3 | 12.5 |

## Conclusion

To sum up, it is concluded that HLA-DRB1*01, *04, *11 and*13 alleles may have a role in susceptibility to the disease, and HLA-DRB1*07 has a protective role against juvenile SLE in the Iranian population. However, there is no significant association between DRB1*08 and SLE in the present study. Our findings could provide useful information for the determination of prognosis in Iranian SLE patients.

**Conflict of interest:** None.

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