BRIEF COMMUNICATION
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Association of HLA-DQB1 Allelic Sequence Variation with Susceptibility to Systemic Lupus Erythematosus

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ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease in which polymorphisms within the human leukocyte antigen (HLA) region have been associated to its etiology. We conducted this study to compare the HLA-DQB1 allelic sequence variation among SLE patients and controls in the northeast of Iran.

Genomic DNA of 40 SLE patients and 83 healthy controls were amplified by Polymerase Chain Reaction with Sequence-Specific Primers technique (PCR-SSP). Seven serological subclasses of the HLA DQB1 were detected.

Allele distribution comparison showed in the SLE group a significant increase of HLA DQ6 (*0601-*0609) (p=0.006); whereas alleles HLA DQ7 (*0301-*0304) were significantly decreased (p=0.005). Combination of DQ5 (*0501-*0504)-DQ6 (*0601-*0609) was increased in patients.

These results suggest that DQ6 is the dominant HLA DQB1 allele probably associated with genetic susceptibility to SLE in the northeast of Iran. The association supports the importance of ethnic background and indicates the importance of various genes that has been observed in different SLE populations.

Key words: Allelic sequence variation; HLA-DQB1; Susceptibility; Systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus is a multifactorial autoimmune disorder characterised by the production of autoreactive T cells and autoantibodies against nuclear, cytoplasmic, and cell surface antigens that may affect every organ system. The more common clinical features seen in patients with SLE include, skin and joint diseases, haematological abnormalities, renal disease and neuropsychiatric complications.[1,2]

Genetic factors together with environmental factors are likely to be important both in determining the overall

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susceptibility to SLE and in influencing the remarkable clinical heterogeneity in disease expression found in affected subjects.[3-5] Several studies have provided interesting insights into the complexity of the genetic interactions involved in SLE but these studies have not yet resulted in the identification of specific genes or genetic pathways mediating SLE susceptibility.[3,4,6-9]

Recent data suggest that the polymorphic genes of the major histocompatibility complex (MHC) may exert its influence in the outcome and expression of the disease, and several HLA-DR or DQ markers or residues, have been implicated in the appearance of specific autoantibodies.[10-15] HLA-DQ antigen is an HLA class II molecule encoded by the 2 sets of DQ genes, DQA1 and DQB1. Although both the DQA1 and DQB1 genes are polymorphic, DQB1 gene is more polymorphic and is the major determinant of the DQ antigen.[3,16,17] Varying interethnic differences in the HLA associations have been reported from different populations around the world.[2,10-15,18]

We conducted this study to compare the HLA-DQB1 allelic sequence variation among SLE patients and controls and analysis of the relative contribution of HLA DQB1 subclasses to the susceptibility to SLE in our patients in the northeast of Iran.

PATIENTS AND METHODS

Systemic lupus erythematosus patients were recruited from the northeastern Iranian region (mainly from the states of Khorasan) between March 2005 and February 2006. These patients were diagnosed at the rheumatology clinic according to the American College of Rheumatology criteria [1]. Forty unrelated patients were genotyped. Mean ages of patients were 24.28±8.21 (with range from 13 to 48) years and the sex ratio (F: M) was 12.3:1. The main symptoms and clinical manifestations they had were nerhritis, arthritis, hematologic abnormalities, malar rash and photosensitivity, oral ulcers, and serositis. The control group consisted of 83 unrelated random healthy individuals. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee at the Mashhad University of Medical Sciences.

HLA-DQB1 Typing

Genomic DNA was isolated from 10cc of anticoagulated whole blood from each patient using DNA extraction kit (Biogene, Mashhad, Iran) by salting-out procedure.[19]

The HLA-DQB1 alleles were determined by a polymerase chain reaction with sequence-specific primers (PCR-SSP) technique. In this procedure seven alleles of HLADQB1 were detected using specific primers in eight PCR reactions. The currently recognized DQB1 alleles were 0501-0504, 0601-0609, 0201, 0301-0305, and 0401-0402. The DQB1 alleles corresponding to the serological specificities DQ4, DQ5 and DQ6 were uniquely identified, whereas the DQ2, DQ7, DQ8 and DQ9 specificities were amplified by two primer mixes.[20,21] For each reaction we used at least 2 primers (Primm-Italy). In total 15 primers were used to identify HLA-DQB1 alleles (Table 1).

Table 1. List of primers used in PCR-SSP method to type HLA DQB1 alleles.

No	Primer name	5`-3` Sequence	Tm (0^c)	Length
1	501LL	GAC-GGA-GCG-CGT-CCG-GGG	66	18
2	315	TGC-AGG-ATC-CCG-CGG-TAC-G	64	19
3	509LL	GGG-ACG-GAG-CGC-GTG-CGT-TA	68	20
4	316	CTG-CAA-GAT-CCC-GCG-GAA-CG	66	20
5	508LL	GGG-ACG-GAG-CGC-GTG-CGT-CT	70	20
6	306L	TGC-AGG-ATC-CCG-CGG-TAC-C	64	19
7	507	GTG-CGT-CTT-GTG-AGC-AGA-AG	62	20
8	307L	TGC-AAG-GTC-GTG-CGG-AGC-T	62	19
9	515LL	GTG-CTA-CTT-CAC-CAA-CGG-GAC-C	70	22
10	308L	GCT-GTT-CCA-GTA-CTC-GGC-GG	66	20
11	309LL	CCA-GTA-CTC-GGC-GTC-AGG-CG	68	20
12	309LV	CAG-TAC-TCG-GCG-GCA-GGC-G	66	19
13	309L	CAG-TAC-TCG-GCG-TCA-GGC-G	64	19
14	516	GCC-GCT-GGG-GCC-GCC-TGA	66	18
15	311LL	CTG-GTA-GTT-GTG-TCT-GCA-TAC-G	66	22

Table 2. Primer mixes for each HLA allele and related genotype.

Primer Mix	Name of Primers	HLA DQB1 Allele	HLA DQB1 Genotype	Size of products (bp)
2	507,307L	2	0201	206
4	515LL,311LL	4	0401,0402	211
5	501LL,315	5	0501-0504	216
6	509LL,508LL,316,306L	6	0601-0609	218,219
7	509LL,309LL,309LV	7	0301-0304	126
2,8	508LL,515LL,308L	2,8	0201,0302	132,147
8,9	508LL,515LL,309L	8,9	0302,0303	125,140
7,9	516,307L	7,9	0301,0303	123

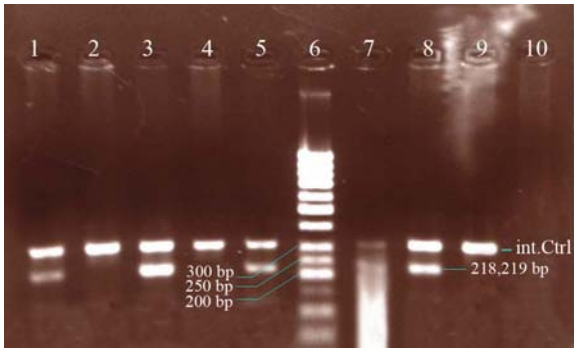

Figure 1. HLA-DQ6 (0601-0609) PCR products as an example of eight PCR mix for typing of seven HLA DQB1 allele group.
Lane 1: positive control,
Lane 2,3,4,5,7,8,9: HLA DQ6 positive and negative subjects,
Lane 6: 50 bp Ladder,
Lane 10: Blank. Internal control.

We used β-actin primers as internal control of PCR in all samples. In HLA DQB1-6 primer mix two pairs of primers were used and the size of products were 218 and 219 bp (Table 2). PCR products were analyzed on 1.5 % agarose gel electrophoresis and documented by gel documentation system (IMAGO, B&L system) as shown (Figure 1).

Statistical Analysis

The strength of statistical association between HLA-DQB1 alleles in patients with SLE was expressed by the relative risk (RR), as presented by Woolf [22]. Intergroup comparison (SLE versus control group) of alleles was examined by the exact test and X^2 test. Significant level was considered to be P less than 0.05.

RESULTS

A significant increase in the frequency of HLA-DQ6 (*0601-*0609) was found among patients with SLE (*P*= 0.006). As is shown (table 3), twenty-five of forty patients with SLE were HLA-DQ6 positive. There was no significant association between DQ6 and severity of disease in these patients. Frequency of DQ7 (*0301-*0304) was found to be decreased in the patient group compared with the controls (*P*= 0.005) (Table 3). There was no significant difference in other alleles among patients and control group.

Approximately twelve patients (30%) showed DQ5 (*0501-*0504)-DQ6 (*0601-*0609) combination vs. seven (8.43%) in controls. The concordance rate of DQ5-DQ6 among SLE patients was significantly higher than control group (*P*= 0.005, RR=4.7).

Table 3. Allele frequency of the HLA-DQB1 in patients with SLE and control groups

DQB1 alleles	Patients N = 40		Controls N = 83		P value
	Number	percentage	Number	percentage	
DQ2 (*0201)	16	40	35	42.17	NS*
DQ4 (*0401- *0402)	1	2.50	2	2.41	NS
DQ5 (*0501-*0504)	17	42.50	35	39.76	NS
DQ6 (*0601- *0609)	25	62.50	27	32.53	P= 0.006 RR= 3.5
DQ7 (*0301- *0304)	4	10	29	34.94	P= 0.005 RR= 0.2
DQ8 (*0302- *0305)	4	10	8	9.64	NS
DQ9 (*0303)	2	5	4	4.82	NS

*NS= non significant means P value>0.05

DISCUSSION

It is universally accepted that genetic factors play an important role in the susceptibility to develop systemic lupus erythematosus and varies among populations from around the world.[3,10-15,23-25] Particular attention has been focused on the association of disease with genetic markers located within the major histocompatibility complex (MHC) region on the short arm of chromosome 6. Several studies have substantially illustrated the fact that SLE is associated with certain MHC alleles in different populations.[2,11-14,18] These studies, however, have been inconclusive in determining a specific MHC gene associated with the disease. The precise genetic cause of the association has been difficult to define because of high degree of polymorphism within the genes of the HLA especially class II antigens [16]. Worldwide reports have found that SLE-HLA association is mainly the result of DQ molecules.[3-5] Among HLA class II antigens HLADQB1 which is associated with several autoimmune diseases, has more polymorphism and is the major determinant of the DQ antigen. Associations have been demonstrated between specific DQB1 polymorphic sequences and various autoimmune diseases such as insulin dependent diabetes mellitus,[26] pemphigus vulgaris[27] and rheumatoid arthritis.[28] Moreover there are several alleles that have been associated with SLE in populations around the world.[6,7,10-15,18,24,25,29,30]

Vargas-Alarcon et al[12] found a relation between DQB1 (*0201) in Mexican SLE patients, Sirikong et al and Morimoto et al.[10,13] determined a significant relation between HLADQB1 *0501 and SLE in Thais and male Japanese patients. Shankarkumar et al,[14] showed a significant increase in the frequency of DQB1*0302, and DQB1*0601 among Indian patients with SLE. HLA-DQB1*0201 and DQB1*0602 were more frequent in Hungarian and also Tunisians SLE patients.[11,24] Some studies in Latin American/Hispanic populations showed that alleles DQB*0201, *0301, and *0602; DQA1*0102 and *0501 have been observed to be associated with SLE.[29] Recently, a comprehensive study was published on HLA class II DNA typing in a large cohort of European patients with SLE.[9]

In our study a significant association between HLA-DQ6 (*0601-*0609) and SLE showed that this allele may be a genetic risk factor for SLE patients in our region. Considering the low frequency of DQ7 (*0301-*0304) in SLE patients versus controls, this allele might play a protective role in susceptibility to SLE.

An interesting result of this study is high frequency of DQ5-DQ6 concordance rate in SLE patients. The association of SLE with various HLA antigens suggests that peptides presented by HLA antigens are numerous, with different affinities of binding sites to pockets of peptide binding grooves of HLA molecules. The results confirm that SLE is a heterogeneous disease and different genetic and environmental factors are responsible for its development. The differences between populations suggest that ethnicity plays an important role in the predisposition for SLE. Thus, studies in other genetically characterized populations are mandatory. In spite of some limitations in our study, including small sample size and using low resolution HLA typing method that influences the power of this study which made firm conclusions difficult, however it can be cautiously concluded that:

SLE-HLA class II association is the result of specific alleles from the three studied loci: HLA-DQ6 (*0601-*0609) being the main SLE susceptibility allele.

Alleles from the DQ5 (*0501-*0504) and DQ6 (*0601-*0609), which are found together probably because of linkage disequilibrium play an important role in susceptibility to systemic lupus erythematosus.

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