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RESEARCH ARTICLE

Molecular analysis of HLA-DRB1 allelic associations with systemic lupus erythematous and lupus nephritis in Egyptians

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Abstract

To evaluate the association of human leukocyte antigen (HLA)–DRB1 alleles with systemic lupus erythematosus (SLE) and lupus nephritis (LN) in the Egyptian population, and to investigate the possible association of HLA-DRB1 alleles with disease severity in LN. HLA-DRB1 alleles were studied in 67 SLE patients (57 patients with LN, 10 patients without LN) and 50 healthy controls by polymerase chain reaction and sequence-based typing assays. The frequency of DRB1*0301 and DRB1*0402 are significantly increased among Egyptian SLE patients compared with the healthy controls. HLA-DRB1*0301, HLA-DRB1*0402 and DRB1*0701 were associated with LN in SLE Egyptian patients. HLA-DRB1*0402 and DRB1*0701 were associated with RFI among SLE Egyptian patients. So we can concluded that, there is an association of an increased risk for SLE in Egyptian with DRB1*0301 and DRB1*0402. The presence of DRB1*0301, DRB1*0402 and DRB1*0701 increase the susceptibility LN in SLE patients. Moreover, DRB1*0402 and DRB1*0701 increase the risk of RFI in those patients.

Introduction

SLE is an autoimmune disease characterized by multiple inflammation and production of autoantibodies to a range of intracellular antigens involved in gene transcription and translation. Genetic factors are likely to be important in both determining the overall susceptibility to SLE and in influencing remarkable clinical heterogeneity in disease expression observed between affected individuals (DCruzet al. 2007). The more common clinical features seen in SLE patients include, skin and joint involvement's, renal involvement, neuropsychiatric complications and also some hematological abnormalities. Genetic factors along with the environmental factors strongly influence the development of SLE. Previous studies both in humans and animal models suggest that SLE is a complex disease with contribution from multiple genes. Multiple loci within the MHC have been implicated in susceptibility like HLA class II alleles, complement components and TNF loci (Eroglu and Kohler 2000).

Human leukocyte antigen (HLA) geneshave received considerable attention among the candidate genes that may be responsible for susceptibility to SLE. It is known that HLA-encoded genes play an important role in SLE susceptibility (DCruzet al. 2007), and many genetic studies have shown that the major histocompatibility complex (MHC), which encodes HLA, isassociated with SLE (Gaffney et al. 2000). In Caucasians, several reportshave shown that the frequencies of HLA-DR2, HLADR3, HLA-A1 and HLA-B8 are significantly increased in SLE patients (Sullivan 2000). DR3 and DR7 were reported to be associated with SLE in Hispanics (Granados et al. 1996) while only DRB1*1503 is significantly increased in African-Americans (Rood et al. 2000). The association of the HLADRB1*1502–DQB1*0501 haplotype with SLE was reported in Thai people(Sirikong et al., 2002) and the DRB1*1501–DQB1*0602 haplotype was reported in Japanese (Hashimoto et al., 1994). Nevertheless, limited information is available concerning whether there is an HLA association with SLE in Egyptians.

Lupus can cause an inflammation of the kidney called lupus nephritis (LN) (DCruzet al. 2007; Morel 2007). About 25–50% of SLE patients develop LN at some time during the course of their illness. LN is a prototypic autoimmune disease manifested by persistent proteinuria, hematuria, swelling of any area of the body, hypertension and/or renal insufficiency. LN is a major cause of morbidity and mortality in SLE patients and can determine the course of SLE. The prognosis of SLE is considerably worse when accompanied by renal involvement.

Although several publications have reported genetic associations with LN in patients from diverse ethnic groups (Tsuchiya et al. 2007; Morel 2007), factors predisposing to development of LN and renal function impairment (RFI) have not been well characterized. The aim of the present study is to use HLA-typing methods to investigate the associations of HLA-DRB1 alleles with SLE and LN in anEgyptian population. Our aim is to determine whether there is an association of HLA-DRB1 alleles with disease severity of LN, in an effort to predict patients at greatest risk of disease progression.

Methods

Patients and Healthy Control

This study was entirely done in Urology and Nephrology Center, Mansoura University. 67 unrelated Egyptian patients (average age 26.9; 55 females, 12 males) with systemic lupus erythromatosous (SLE) were included in this study. All patients met the American Rheumatism Association criteria for SLE (Doria et al 1994), and were being followed at nephrology clinics at the Urology and Nephrology Center between 2008 and 2013.

Patients were determined to have lupus nephritis (LN) (n=57) according to the standard diagnostic criteria (Doria et al 1994), if they had persistent proteinuria exceeding 500 mg daily. LN patients were subdivided into two groups, based on whether there was renal function impairment (RFI) at diagnosis: the RFI group (n=23) had elevated serum creatinine (Cr > 1.4 mg/dL), while the non-RFI group (n=34) had clinical evidence of LN without RFI.

The control group consisted of 50 healthy renal donors (41 female, 9 males) who were ethnically matched, resided in the same area and had no personal history of autoimmune disease. Written informed consent was obtained from all subjects and the study was approved by the Institutional Review Board of Urology and Nephrology Center.

Sampling

Blood (3-4 ml) was collected in EDTA tubes and stored in the freezer (-20°C), and it was used for DNA extraction. Plasma samples obtained after centrifugation (3000 RBM for 10 min) were used for determination of creatinine. Twenty four urine samples were collected in polypropylene containers for determination of urinary protein.

Molecular determination of HLA class II DRB*1:

DNA Isolation:

Genomic DNA was isolated from 200 µl of whole blood by using the silica membrane spin column based kit (QIAGEN, Hilden, Germany) according to manufacturer's.

Principles of HLA-DRB1 Genotyping (Inno-LiPA HLA Typing):

The Inno-LiPA HLA typing test is based on the reverse hybridization principle, in which amplified biotinylated DNA material is chemically denatured, and the separated strands are hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. This is followed by a stringent wash step to remove any mismatched amplified material. After that, streptavidin conjugated with alkaline phosphatase is added and bound to any biotinylatedhybrid previously formed. Incubation with a substrate solution containing a chromogen results in a purple/brown precipitate. The reaction is stopped by a wash step, and the reactivity pattern of the probes is recorded.

DNA Amplification of HLA-DRB1 Gene:

The DNA amplification was carried out using the Inno-LiPA HLA-DRB1 Multiplex kit (Innogenetics, Belgium), which is intended for nucleic acid amplification of the second exon of HLA-DRB1 locus. The reaction is performed by means of polymerase chain reaction (PCR) in a multiplex format.

Molecular Typing of HLA-DRB1 alleles by Inno-LiPA:

As summarized in the principles of test, the amplification products were subsequently hybridized using one HLADRB1 typing strip (Innogenetics, Belgium) for each sample, on which 37 sequence-specific probe lines and 2 control lines are fixed. The HLA-DRB1 genotype was determined using the computer package Interpretation software LiRAS™.

Statistical analysis

Data analysis and transformation, t-test, one way analysis of variance (ANOVA) were done using IBM compatible PC, SPSS/PC+ package.

Results

Patient characteristics

Patient characteristics are summarized in **Table 1**. Among the 67 SLE patients, 57 (85%) were diagnosed with LN, if they had proteinuria exceeding 500 mg daily. Roughly 83.3% (10/12) of male SLE patients had LN, and 85.4% (47/55) of female

SLE patients had LN. Overall, 42.6% (20/47) of female LN patients had RFI, whereas only 30% (3/10) of male LN patients had RFI.

HLA-DRB1 allele frequencies in SLE patients and controls

The distribution of the DRB1 in SLE patients and controls was summarized in **Table 2**. As illustrated, the more frequent genotypes in controls were DRB1*1101 (14%), DRB1*1301 (12%), DRB1* 0401 (12%) and DRB1*0701 (11%). In SLE group, the major genotypes of DRB1 were DRB1*0301(24.1%), DRB1*0402 (19%) and DRB1*0701(14.7%). The frequencies of DRB1*0301 and DRB1*0402 were significantly increased ($P<0.001$; $P<0.01$, respectively) in SLE patients when compared with the controls. However, the frequencies of DRB1*0401($P<0.001$), DRB1*1101 ($P<0.01$) and DRB1*1301 ($P<0.01$) were significantly decreased in the SLE patients vs. controls. There were no significant differences in the frequencies of the other DBR1 alleles between the two groups.

HLA-DRB1 allele frequencies in SLE patients with and without nephritis

The frequencies of the DRB1 in SLE patients with and without nephritis were summarized in **Table 3**. As shown, the frequencies of DRB1*0301 ($P<0.001$), DRB1*0402 ($P<0.001$) and DRB1*0701 ($P<0.01$) were significantly increased in SLE patients with nephritis when compared with those without nephritis.

HLA-DRB1 allele frequencies in nephritis patients with and without RFI

The distribution of the DRB1 in nephritis patients with and without RFI was summarized in **Table 4**. As illustrated, the frequencies of DRB1*0402 and DRB1*0701 were significantly decreased ($P<0.01$) in nephritis patients with RFI when compared with those without RFI.

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Table 1 Patient and control characteristics

	Control	SLE	SLE without LN	LN	LN with RFI	LN without RFI
n	50	67	10	57	23	34
Sex						
Female	41	55	8	47	20	27
Male	9	12	2	10	3	7
Age (yr)	28.3±6.5	26.8±8.2	26.4±9.0	26.7±7.9	26.9±7.4	26.8±8.7
Creatinine (mg/dL)	0.79±0.19	2.24±2.61	0.82±0.41	2.18±2.84	4.51±2.92	0.83±0.30
Proteinuria (g/day)	0.22±0.09	3.97±3.60	0.38±0.10	4.45±3.39	4.72±4.03	3.49±3.27

Table 2 Allele frequencies of the DRB1 gene in patients with SLE and controls

DRB1 allele	The frequency %	
	The control group	The SLE group
DRB1*0101	5.0	3.4
DRB1*0102	1.0	4.3
DRB1*0301	9.0	24.1**
DRB1*0302	4.0	0.0
DRB1*0303	1.0	0.0
DRB1*0401	12.0	0.9**
DRB1*0402	7.0	19.0*
DRB1*0701	11.0	14.7
DRB1*0702	1.0	0.0
DRB1*0801	1.0	0.9
DRB1*1001	3.0	5.2
DRB1*1101	14.0	6.0*
DRB1*1102	6.0	5.2
DRB1*1156	2.0	0.0
DRB1*1301	12.0	3.4*
DRB1*1302	4.0	0.9
DRB1*1501	7.0	0.9
DRB1*1104	0.0	0.9
DRB1*1303	0.0	5.2
DRB1*1401	0.0	1.7
DRB1*1404	0.0	1.7
DRB1*1502	0.0	1.7

*p<0.01;
**p<0.001

Table 3 HLA-DRB1 allele frequencies in SLE patients with and without nephritis

DRB1 allele	The frequency %	
	SLE with nephritis	SLE without nephritis
DRB1*0101	3.5	-
DRB1*0102	3.5	0.86
DRB1*0301	21	2.6**
DRB1*0401	1.7	-
DRB1*0402	18	0.86**
DRB1*0701	12	2.6*
DRB1*0801	0.86	-
DRB1*1001	4.3	0.86
DRB1*1101	6	-
DRB1*1102	5	-
DRB1*1301	2.6	-
DRB1*1302	-	0.86
DRB1*1501	0.86	-
DRB1*1104	0.86	-
DRB1*1303	5	-
DRB1*1401	1.7	-
DRB1*1404	0.86	1.86
DRB1*1502	1.7	-

*p<0.01;
**p<0.001

Table 4 HLA-DRB1 allele frequencies in nephritis patients with or without RFI

DRB1 allele	The frequency %

	LN with RFI	LN without RFI
DRB1*0101	1.8	1.8
DRB1*0102	2.6	1.8
DRB1*0301	12.3	14
DRB1*0401	-	0.88
DRB1*0402	4.4	14.9*
DRB1*0701	5.3	9.5*
DRB1*0801	-	0.88
DRB1*1001	2.6	2.6
DRB1*1101	2.6	3.5
DRB1*1102	3.5	1.8
DRB1*1301	1.8	1.8
DRB1*1302	-	0.88
DRB1*1501	0.88	-
DRB1*1104	-	1
DRB1*1303	2	4
DRB1*1401	-	2
DRB1*1404	2	-
DRB1*1502	1	1

*p<0.01

Disscusion

The precise etiology of SLE remains unknown, but many studies have revealed that genetic and environmental factors are implicated in the development of SLE. However, the genetic basis for the susceptibility to the disease or for the activity of the disease has yet to be fully explained. The immunological abnormalities are considered to be responsible for the pathogenesis of SLE. Therefore, the aim of the present study is to use HLA-typing methods to investigate the associations of HLA-DRB1 alleles with SLE and LN in an Egyptian population.

For this purpose, 67 SLE Egyptian patients were investigated. This group was divided into two groups (SLE with LN and SLE without LN) according to if they had persistent proteinuria exceeding 500 mg daily. The LN group is subdivided into two groups (LN with RFI and LN without RFI) according to the serum creatinine level. All patients as well as 50 matching healthy persons were subjected to molecular determination of HLA class II DRB*1.

One of the major findings of this study are that the frequency of DRB1*0301 and DRB1*0402 are significantly increased among Egyptian SLE patients and that DRB1*0401, DRB1*1101 and DRB1*1301 were negatively associated with SLE patients. The previously studies revealed that HLA associations with SLE are discrepant in the allelic distribution for different ethnic groups.

An association between HLA-DRB1*1502 and SLE has been reported in Thai (Sirikonget al. 2002) and Japanese populations (Hashimoto et al. 1994). Pan et al. (2009) found an association of an increased risk for SLE in Taiwanese with DRB1*0301 and DRB1*1501. There are three haplotypes strongly linked to development of SLE in Caucasians: 1) HLA-DRB1*1501(DR2)-DQB1*0602, 2) HLA-DRB1*0301(DR3)-DQB1* 0201 and 3) HLA-DRB1*0801(DR8)-DQB1*0402 (Wong and Tsao 2006). DRB1*0301 is the principle allele associated with the genetic susceptibility to SLE in Mexican patients (Vargas-Alarcon et al. 2001).

Since LN is a major cause of morbidity in SLE patients and determines the prognosis of SLE, this finding may provide clinically useful information for determining the prognosis of SLE patients.

Our study revealed that HLA-DRB1*0301, HLA-DRB1*0402 and DRB1*0701 were associated with LN in SLE Egyptian patients.

Several prior studies have investigated whether specific alleles are associated with LN in SLE patients. HLA class II antigens appear to associate with LN in Italian SLE patients (Marchiniet al. 2003). HLA-DRB1*03 was associated with kidney involvement in European SLE patients (Galeazziet al. 2002) and HLA-DRB1*1503 was associated with worsening proteinuria in a population of largly Hispanic patients (Bastian et al. 2007). Frequencies of HLA-DRB1*17, DRB1*10, DRB1*15 and DRB1*07 alleles were all significantly elevated in juvenile-onset SLE patients with nephritis (Liphauset al. 2007).

The mechanism by which HLA alleles influence susceptibility to SLE and LN is still unknown, although SLE autoantibodies may play a role. Anti-RO and anti-La were associated with DQB1*0201 and DRB1*03 while anti-dsDNA was associated with DRB1*15(Galeazziet al. 2002).

Most SLE patients show positive anti-dsDNA and the majority of patient with LN are anti-dsDNA positive. Immune complexes consisting of anti-dsDNA can be deposited in the kidney and this may lead to a local inflammatory process (Bao and Quigg 2007).

Evaluating renal function in SLE patients is important because early detection and treatment of renal involvement can significantly improve renal outcome.

It has been suggested that certain MHC haplotypes show stronger association with SLE clinical manifestations. For example, Liphaus et al. 2007 observed that the HLA-DRB1*15 allele was most frequent in patients with cutaneous, cardiac, neuropsychiatric, renal, and hematologic involvement.

Doherty et al. 1992 described an association with HLA-DR15 and SLE skin involvement in Chinese patients and Reivelle et al. 1991 demonstrated an association with HLA-DR53 and HLA-DQA1 and malar erythema photosensitivity. They also reported an association with the HLA-DR7 and HLADR9 alleles and cardiac involvement in Chinese patients.

The present study revealed that HLA-DRB1*0402 and DRB1*0701 were associated with RFI among SLE Egyptian patients. This indicates that these two alleles may contribute to RFI in SLE patients.

In conclusion, we found an association of an increased risk for SLE in Egyptian with DRB1*0301 and DRB1*0402. Results from our study also suggest that DRB1*0401, DRB1*1101 and DRB1*1301 are negatively associated with SLE. The presence of DRB1*0301, DRB1*0402 and DRB1*0701 increase the susceptibility LN in SLE patients. Moreover, DRB1*0402 and DRB1*0701 increase the risk of RFI in those patients. These results could provide useful information for the determination of prognosis in Egyptian SLE patients.

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