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ORIGINAL ARTICLE

Association of HLA-DQB1*06 with susceptibility to systemic lupus erythematosus in Egyptians

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Abstract *Aim of the work:* This study was conducted to compare the HLA-DQB1*06 allele frequency among Egyptian SLE patients and controls. The association of that allele with clinical features and distinct autoantibody profiles in SLE patients was also assessed.

Patients and methods: This study included 48 consecutive Egyptian SLE patients and 49 age and sex matched unrelated healthy Egyptian subjects as a control group. All patients underwent full history taking, thorough clinical examination, SLE cumulative organ damage was scored using the Systemic Lupus International Collaborating Clinics (SLICC) damage index. Routine laboratory investigations were done as well Genomic DNA amplification by polymerase chain reaction with sequence-specific primers technique (PCR-SSP) to detect HLA DQB1*6.

Results: HLA-DQB1*06 was significantly more frequent among the SLE patients. Our results showed HLA-DQB1*06 to be associated with SLE (39.6% in patients and 8.2% in controls, OR = 7.23; 95% CI = 2.22–23.6; P = .00). We found no association with the clinical findings or antibodies found in our patients apart from a significant negative association with vasculitis.

Conclusion: The current work suggests that DQB1*06 allele is a susceptibility allele in Egyptian patients with SLE but is not related to the clinical presentation or laboratory tests of SLE. To our knowledge this is the first study of the frequency of DQB1*06 alleles in Egyptian SLE patients.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with variable clinical presentation. SLE can affect all organs and the involvement of major organs can be life threatening. The exact pathological mechanisms of SLE remain elusive, and the etiology of SLE is known to be multifactorial [1]. The disease is due to the interaction between genetic, immunologic, endocrine and environmental factors [2]. Therefore, gene–gene and

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gene–environment interactions that combine to cause the disease complicate the interpretation of the data generated from family-based linkage and association studies [3]. Recent genome wide association studies have identified many candidate genes for lupus susceptibility [4].

To date, the strongest associations of genetic factors with SLE are with variants located in the major histocompatibility complex (MHC) on the short arm of chromosome 6 that contains hundreds of genes, many of them with immune-related functions [5,6]. The MHC class II region is the densest area of the genome for disease associations [7]. The MHC can be divided into three regions, termed classes I, II, and III. Classical classes I and II loci encode the human leukocyte antigen (HLA) proteins involved in antigen presentation to T cells [6]. The centromeric Class II region contains the highly polymorphic HLA-DR, -DQ and -DP genes. Class II molecules are heterodimers consisting of an alpha (DQA) and a beta chain (DQB), both anchored to the membrane, which play a central role in the immune system by presenting peptides derived from extracellular proteins. These molecules are expressed at high levels on antigen-presenting cells, such as dendritic cells and B cells and display peptides for CD4 helper T cells. These genes have been found to consistently associate with SLE in several populations [8,9]. 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 [10]. An association between HLA-DQ6 and SLE patients among some races in the Middle East has been reported.

The degree of association between SLE and specific genes of the MHC region varies considerably from one population group to another [11,12]. Understanding the genetic basis may predict high-risk patients who carry one or more genetic susceptibility factors among the general population or within families. In addition, if we know the environmental factors or triggering factors of the disease, the identification of individuals with high-risk genetic background may prevent or at least delay the development of disease by the environmental control and genetic counseling of the high-risk family members as well as enhance our understanding of disease pathogenesis [13]. In the future, knowledge of an individual's genotype may help us tailor the most appropriate treatment for that SLE individual [3].

Aim of the work: We conducted this study to compare the HLA-DQB1*06 allele frequency among SLE patients and controls and analyze its contribution to the susceptibility to SLE in Egyptians. In addition, we assessed the association of that allele with clinical features of the disease and distinct autoantibody profiles in SLE patients.

2. Patients and methods

This study included 48 consecutive Egyptian SLE patients recruited from the Departments of Rheumatology and Rehabilitation Cairo and Ain Shams Universities, Egypt. Diagnosis of SLE was established according to the American College of Rheumatology (ACR) revised Criteria for diagnosis of SLE [14]. The study also included 49 age and sex matched unrelated healthy Egyptian subjects as a control group. The nature of the present study and a written informative consent was obtained from all patients, which was approved by the local Ethics Committee. Exclusion criteria included non-Egyptian patients, related SLE patients, and patients with other autoimmune disease or overlap syndrome.

2.1. Patient evaluation

SLE patients were subjected to thorough history taking, general and local examination. The SLE cumulative organ damage was scored using the Systemic Lupus International Collaborating Clinics (SLICC) damage index [15]. Laboratory investigations included full blood picture, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum creatinine, simple urine analysis and estimation of 24 h urinary protein, urine protein/creatinine ratio (P/C ratio), alanine transaminase (ALT), aspartate transaminase (AST), C3, C4, antinuclear antibody (ANA) and anti-double stranded deoxyribonucleic acid (anti-dsDNA). Blood and urine samples were collected on the same day. Radiological studies including plain X-ray of the chest, affected joints and echocardiography when needed.

2.2. Determination of HLA-DQB1*06 gene

DNA was extracted from white blood cells of 2 mL of k3 EDTA samples by column extraction method using QIA amp DNA blood mini kit-Qiagen (from HOFFmann-la Roche AG). HLA-DQB1Gene was amplified and detected by polymerase chain reaction with sequence specific primers (PCR-SSP) technique. Each PCR reaction mixture contained four group specific DQB1 primers as well as the internal control primer pair, to avoid false-negative typing results due to primer competition. The internal positive control primers were included in a fourfold lower concentration than the allele and group-specific primers. Their sequences were (forward primer 5′GCCTTCCCAACCATTCCCTTA3′), (reverse primer 5′TCACGGATTTCTGTTGTGTTTC3′) (Table 1). Each 10 μL PCR reaction consisted of 100 ng genomic DNA, PCR buffer 50 mM KCl 1.5 mM^{-1} $MgCl_2$ 10 mH^{-1} Tris–Cl,

Table 1 Nuclotide sequences, length, Tm and specificities of the PCR primers used for identification of the allelic series of DQ series by the PCR-SSP technique.

Primer *mix*[2]	Primer sequence	Length	T_m	Size of PCR product	Amplified specificities
DQ6	Forward Sequence			218/219 bp	DQB1
	5′GGGACGGAGCGCGTGCGTTA3′	20 mer	68 °C		
	5′GGGACGGAGCGCGTGCGTCT3′	20 mer	70 °C		
DQ6	Reverse Sequence				601–609
	5′CTGCAAGATCCCGCGGAACG3′	20 mer	66 °C		
	5′TGCAGGATCCCGCGGTACC3′	19 mer	64 °C		

pH $\frac{8.3}{0.001}$% (w/v)gelatin), 200 μm each of d ATP, d GTP and d TTP 0.40 μm of the allele and group. Specific DQB1 primers, 0.10 μm of the control primers and 0.25–0.40 units of Ampli Taq (perkin–Elmer cetus corporation), 5% (v/v) glycerol (redistilled) [16,17] and cresol red, sodium salt (100 μ mL^{-1}). PCR amplification was carried out in a thermal cycler. After an in initial denaturation at 94 °C for 2 min. DNAs were amplified by 30 amplification cycles. The first 10 cycles consisted of denaturation at 94 °C for 10 s and a combined annealing-extension step at 66 °C for 60 s, the 20 following cycles consisted of denaturation at 94 °C for 10 s, annealing at 61 °C for 50 s and extension at 72 °C for 30 s. The same PCR parameters were used for conventionally as well as for rapidly extracted DNAs.

The whole PCR reactions were directly electrophoresed in 2% ME agarose gels prestained with eithidium bromide (0.25 μg mL^{-1} geL) the gels were run for 15 min at 10 cm^{-1} in 0.5× TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8) and visualized under UV illumination (Fig. 1).

Statistical methods: IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. Data were expressed as Median & Percentiles for quantitative non-parametric measures and in addition, both number and percentage for categorized data.

The following tests were done:

1. Chi-square test to study the association between each of the 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 are highly significant.
2. Calculated Relative Risk Assessments (Relative Risk Ratio or RRR) that measure how many times the risk was present among diseased individuals as that among non-diseased ones. They were calculated as absolute figures and as a standard error of estimate (95P).

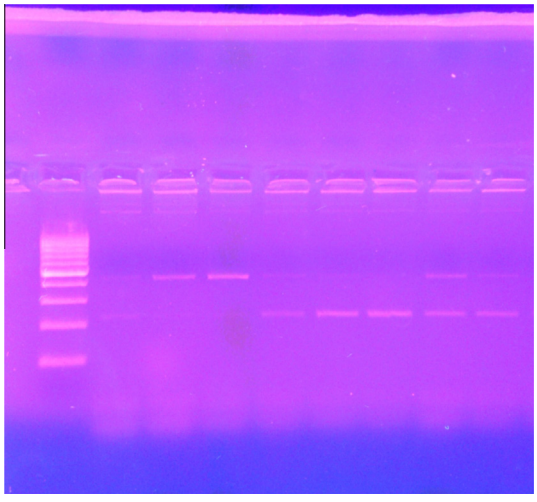

Figure 1 HLA-DQ6 (601–609) PCR products as an example of eight PXR mix for typing of seven HLA DQBI allele group. Positive subjects show 218,219 bp bands and negative subjects show no bands at 218,219 bp. Lane 1: 100 bp Ladder. Lanes: 2, 5, 6, 7, 8, 9 HLA DQ6 positive subject. Lanes: 3, 4 negative subject.

3. Results

This study included 48 SLE patients; their ages ranged from 18 to 51 with a mean of 31.37 ± 8.85 years. Their disease duration ranged from 0.5 to 35 years with a mean of 10.96 ± 8.64 years. Our patients were 42 females and 6 males with a female to male ratio of 7:1. The study also included 49 age and sex matched healthy subjects that served as a control group. Clinical presentation and laboratory data of SLE patients are shown in Table 2. The SLICC score in our patients ranged from 0 to 2.

HLA-DQB1*06 was significantly increased in patients versus controls denoting a positive association between it and SLE in Egyptian patients (Table 3). It failed to show any association with the clinical findings or antibodies found in our patients apart from a significant negative association with vasculitis (Table 4). The age of onset of SLE did not show significant difference among patients positive for HLA-DQB1*06 and patients lacking this allele ($P > 0.05$). 73.10% of the HLA-DQB1*06 negative patients had a SLICC score of 0, 23.10% had a score of 1 while 3.80% had a score of 2. The HLA-DQB1*06 positive patients showed 61.10% with a SLICC score of 0, 38.90% with a score of 1 while none of the patients scored 2. The presence of the allele did not associate with the SLICC score (OR = 1.34, $P = 0.403$).

4. Discussion

Systemic lupus erythematosus (SLE) is a serious prototype autoimmune disease characterized by chronic inflammation, autoantibody production and multi-organ damage [9]. Genetic susceptibility to SLE is well established. MHC is the strongest genetic factor for SLE. Altered frequencies of human leukocyte antigen (HLA) alleles are known to be associated with

Table 2 Clinical presentation and laboratory data of the systemic lupus erythematosus (SLE) patients.

Clinical presentation	Number (%)
Mucucutaneous manifestations	37 (77.1)
Musculoskeletal manifestations	37 (77.1)
Serositis	22 (45.8)
Cardiac affection	3 (6.25)
Pulmonary affection	5 (10.4)
CNS affection	15 (31.25)
GIT affection	5 (10.4)
Hematologic affection	25 (52.1)
History of DVT	9 (18.75)
Renal affection	29 (60.4)
Raynaud's phenomenon	4 (8.3)
Vasculitis	11 (22.9)
Laboratory data	
Positive ANA	42 (87.5)
Positive anti dsDNA	37 (77.1)
Lupus Anti Coagulant	12 (25)
Positive Antiphospholipid	12 (25)
Positive anti cardiolipin	14 (29.2)

CNS: central nervous system, GIT: gastrointestinal, DVT: deep venous thrombosis, ANA: antinuclear antibody, dsDNA: double stranded deoxyribonucleic acid.

Table 3 Frequency of HLA-DQB1*06 in systemic lupus erythematosus (SLE) patients versus healthy controls.

HLA-DQB1*06	Control		SLE patients		OR	95% CI	*P*-value	Sig.
	N	(%)	N	(%)				
Negative	45	(91.8)	29	(60.4)	7.23	2.22–23.6	0.000	HS
Positive	4	(8.2)	19	(39.6)				

HLA, human leucocyte antigen; OR, odds ratio; 95% CI, 95% confidence interval.
*Significant *P*-value if <0.05, highly significant *P*-value if <0.01.

Table 4 HLA-DQB1*06 genotype frequency in SLE patients relative to SLE phenotype.

Manifestation		HLA-DQB1*06 in SLE patients (%)		OR	95% CI	*P*	Sig
		Negative	Positive				
Mucocutaneous	Neg.	25	11.1	2.7	0.49–14.6	0.25	NS
	Pos.	75	88.9				
Musculoskeletal	Neg.	14.3	27.8	0.43	0.09–1.9	0.26	NS
	Pos.	85.7	72.2				
Serositis	Neg.	60.7	38.9	2.43	0.72–8.18	0.15	NS
	Pos.	39.3	61.1				
Cardiac	Neg.	92.9	94.4	0.77	0.06–9.11	0.83	NS
	Pos.	7.1	5.6				
Pulmonary	Neg.	89.3	88.9	1.04	0.16–6.94	0.97	NS
	Pos.	10.7	11.1				
CNS	Neg.	64.3	72.2	0.69	0.19–2.51	0.58	NS
	Pos.	35.7	27.8				
GIT	Neg.	89.3	88.9	1.04	0.16–6.94	0.97	NS
	Pos.	10.7	11.1				
Hematologic	Neg.	53.6	33.3	2.31	0.68–7.89	0.18	NS
	Pos.	46.4	66.7				
History of DVT	Neg.	85.7	72.2	2.31	0.53–10.1	0.26	NS
	Pos.	14.3	27.8				
Renal	Neg.	39.3	33.3	1.29	0.38–4.4	0.68	NS
	Pos.	60.7	66.7				
Raynaud's	Neg.	85.7	100	–	–	0.09	NS
	Pos.	14.3	0				
Vasculitis	Neg.	63	94.4	0.09	0.01–0.78	0.02	S
	Pos.	37	5.6				
Positive ANA	Neg.	14.3	0	–	–	0.09	NS
	Pos.	85.7	100				
Positive antidsDNA	Neg.	25	11.1	2.7	0.49–14.6	0.25	NS
	Pos.	75	88.9				
LA	Neg.	75	70.6	1.15	0.30–4.41	0.75	NS
	Pos.	25	29.4				
Positive APL	Neg.	75	70.6	1.15	0.30–4.41	0.75	NS
	Pos.	25	29.4				
Positive ACL	Neg.	75	58.8	1.91	0.53–6.84	0.26	NS
	Pos.	25	41.2				

LA: lupus anticoagulant, APL: antiphospholipid, ACL: anticardiolipin.

SLE [18], with the HLA class II DRB1 and DQB1 loci demonstrating the strongest association [19].

Our results showed HLA-DQB1*06 to be associated with SLE (39.6% in patients and 8.2% in controls, OR = 7.23; 95% CI = 2.22–23.6; $P = 0.000$). Similar results were reported among Saudi SLE patients where the HLA-DQB1*06 frequency was 40% (OR = 1.67; 95% CI = 1.19–2.36; $P = 0.003$) [20]. A significant increase in the frequency of HLA-DQ6 (*0601–*0609) was found among Iranian patients with SLE where 25 of 40 patients (62.5%) with SLE were HLA-DQ6 positive. This was the highest frequency compared to other DQB1 alleles (DQ2, DQ4, DQ5, DQ7, DQ8 and DQ9) [10]. Frequencies of DQB1*0602 were also significantly increased amid Tunisians among other HLA alleles (DRB1*1501–DQA1*0102, and DRB1*0301–DQA1*0501–DQB1*0201) [21]. Shankarkumar et al. showed a significant increase in the frequency of DQB1*0601, among other alleles in Indian patients with SLE [22]. HLA-DQB1*06 was also present in 23% of Hungarian SLE patients [23].

The role of HLA molecules in autoimmune disease might be related to their role in antigen presentation to T cells, in terms of presentation of a disease-triggering self-peptide, or altered-self-peptide. Alternatively, HLA may play a causative role via influence on the T-cell repertoire, including T

regulatory cells, resulting in potential autoreactivity. Finally, the associated HLA polymorphism may play no direct role, merely acting as a marker [8,24–26].

Studies in other populations gave varying results. Vargas-Alarcon et al. reported that the DRB1*0301 is the principal class II allele associated with the genetic susceptibility to SLE in Mexicans [27]. Similar results were reported in Latin Americans with no HLA-DQB1 alleles showing a significant deviation from the control group [2,28]. Sirikong et al. in 2002 reported a significant association of HLA-DRB1*1502–DQB1*0501 haplotype was demonstrated in Thais [12]. Among Hungarian SLE patients there was increased prevalence of the DRB1*1501, DRB1*03 DQA1*0102, DQB1*0201, as well as DQB1*0602 alleles [29]. Studies on European ancestry to determine association at classical HLA genes showed association with HLA-DRB1*03:01, HLA-DRB1*08:01, and HLA-DQA1*01:02 [6].

The possible explanations for differences of susceptible genetic factors between populations could be different genetic backgrounds, contribution of gene–gene or gene–environment interaction, and the relation between marker and causal variants. Therefore, efforts to identify ethnic-specific genetic factors or disease causing variants are necessary for individualized therapy for SLE in future [13].

We also explored associations of DQB1*06 alleles with SLE phenotypes including the presence of autoantibody and clinical manifestations of disease. We found no association with the clinical findings or antibodies found in our patients apart from a significant negative association with vasculitis. Rezaieyazdi et al. [10] also reported absence of significant association between DQ6 and severity of disease in their patients. Contrary to our results Malaysians demonstrated significant association of DQB1*0601 with anti-Sm/RNP and with anti-dsDNA [30]. Tunisians showed no significant association of HLA-DQB1*06 with any of the specific antibodies in SLE [21].

In conclusion, the current work suggests that the DQB1*06 allele is a susceptibility allele in Egyptian patients with SLE but is not related to the clinical presentation or laboratory tests of SLE. To our knowledge this is the first study of the frequency of DQB1*06 alleles in Egyptian SLE patients.

Conflict of interest

We have no conflict of interest to declare.

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