

## **Relation between HLA typing and clinical presentations in Systemic Lupus Erythematosus patients in Al-Qassim region, Saudi Arabia**

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### **Abstract**

**Background:** Systemic lupus erythematosus (SLE) is a disease with diverse clinical presentations due to interaction between genetic and environmental factors. SLE is associated worldwide with polymorphisms at various loci, including the major histocompatibility complex (MHC), although inconsistencies exist among these studies.

**Aims:** This study was carried out to investigate, the association of HLA-DRB1, DRB3, DRB4, DRB5, and DQB1 alleles in SLE patients and clinical presentations at Qassim, Saudi Arabia.

**Methods:** Fifty one patients with SLE—84.3% of whom had kidney involvement were studied in a case control study for HLA-DRB1, DRB3, DRB4, DRB5, and DQB1.

**Results:** It was found that DRB3 is a protective gene among Saudi's against SLE, HLA DRB3, HLA DRB1\*11 frequency was increased in patients with serositis with a p value of (0.004), (0.047) respectively, increased frequency of HLA DQB1\*3 among SLE patients with skin manifestations with a p value of (0.041), the frequency of HLA DRB1\*15 alleles was increased among SLE patients with nephritis with a p value of (0.029), the frequency of HLA DRB1\*11 among those with hematological manifestations with a p value of (0.03) and the frequency DRB1\*10 was found to be increased among SLE patients with neurological manifestations with a p value of (0.002)

**Conclusion:** In contradistinction to what have been found among other populations DRB3 is a protective gene among Saudi's against SLE. No evidence for a role of the HLA-DRB1, DRB4, DRB5, DQB1 alleles. There was an increased HLA DRB3 frequency with serositis, DQB1\*3 skin manifestations, HLA DRB1\*15 with nephritis, DRB1\*10 with hematological manifestations and DRB1\*11 with neurological manifestations.

**Key words:** SLE; HLA; Saudi; disease clinical expression; lupus

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## Introduction

Systemic lupus erythematosus (SLE) is a clinically heterogeneous inflammatory disorder characterized by multi-systemic organ involvement and the production of auto antibodies against a range of intracellular, cell surface and serum components. Diverse genetic and environmental factors are implicated in its etiology.<sup>(1-4)</sup> The association between SLE and polymorphisms at several loci, including the major histocompatibility complex (MHC), which encodes human leukocyte antigen (HLA), complement proteins, immunoglobulin receptors, cytokines, and other unmapped genes, has been stated by several genetic studies.<sup>(5, 6)</sup> The genetic role of MHC is well studied, and association<sup>(7)</sup> as well linkage studies<sup>(8)</sup> undoubtedly demonstrated the presence of SLE susceptibility factors in the MHC region. However, this relationship's nature is obscured, as the degree of association between SLE and specific genes of the MHC region varies considerably from a particular population to another.<sup>(9, 10)</sup> One of the most accordant findings is the increased frequency of HLA-DR2 (HLADRB1 \*15 and HLA-DRB1 \*16),<sup>(11, 12)</sup> HLADRB1 \*03, 11 and in particular the haplotype HLA-A \*01, B \*08, DR3 in Caucasian SLE populations.<sup>(13, 14)</sup> And in Malaysia HLA A\*1101, 1102, DRB5\*01-02, DQB1\*05, DRB3\*0101, 0201, 0202, 0203, 0301, and DQB1\*0301, 0304 were significantly associated with SLE.<sup>(4)</sup> While Elsherbini et al found that DR4 and DR13 are associated with SLE among the Egyptians.<sup>(15)</sup> Despite the considerable progress made in the last several years towards elucidation of the genetic basis of susceptibility to SLE, the markers remain to be ill-identified, a thing which is in accordance with the known polygenic basis of the disease, reflecting diversity of contributions from multiple genes. While most of the studies have looked for an association between HLA alleles and SLE, few have tried to look at the relationship between these markers and SLE manifestations, severity, and clinical and serological subsets. The aim of this study was to investigate the association between HLADRB1, DRB3, DRB4, DRB5, and DQB1 alleles and SLE in Qassim region, Saudi Arabia.

## Subjects and Methods

This is a case control study in which fifty one SLE patients, forty seven of which were Saudis with (2 males and 49 females) were recruited and followed up in an outpatient clinic of the Rheumatology Unit, King Fahad Hospital of Buraidah, a Ministry of Health tertiary level hospital. The diagnosis of SLE was made according to the American College of Rheumatology (ACR) criteria. The definitions for the presence of the other clinical characteristics, such as nephropathy and others like the hematological abnormalities, arthritis, and the rest of the clinical manifestations were also based on the ACR criteria and according to a standard protocol. Severity of disease was judged using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Thirty healthy antinuclear antibody negative volunteers were studied as controls. Patients and controls were recruited after signing an informed consent from the same geographic area of Qassim, and all were of Arab origin except one. Peripheral blood samples (10 mL) were collected in Ethylene diamine tetra-acetic acid (EDTA). Genomic DNA was extracted from proteinase-K-treated peripheral blood leukocytes by using a Salting-Out procedure.<sup>(16)</sup> DNA was amplified using polymerase chain reaction (PCR) and sequence-specific primers (PCR-SSP).<sup>(17)</sup>

## DNA Extraction

Two ml of whole blood were mingled with 8 ml of triton lysis buffer 1 (0.32M Sucrose, 5mM MgCl<sub>2</sub>.6H<sub>2</sub>O, 12mM Tris-HCl, pH 7.5, 1%V/V Triton X-100). Leukocytes and nuclei were stirred up (3500g, 5min), the pellet was washed with dH<sub>2</sub>O and then restirred in 0.9 ml of lysis buffer 2 (0.375M NaCl, 0.12M EDTA, pH 8.0), 25 µl SDS 10%, and 0.22 ml NaClO<sub>4</sub> (4M) and was waggled actively, spun down (13000g, 5 min) and consequently salted out with a saturated NaCl solution. DNA in the supernatant was precipitated with 99.5% ethanol. Finally, DNA pellet was deliquesced in 100 µl of ddH<sub>2</sub>O. DNA was quantitated using UV spectrophotometry, and then 100ng of genomic DNA was used for each 20 µl PCR reaction.

### DNA amplification and detection

For HLA-DR low resolution typing by PCR-SSP, 14 independent reactions were done per sample: ten for assigning HLA-DRB1\* 01, 03, 04, 07, 09, 10, 11,12,13,14, 15, 16, DRB3, 4, 5 and DQB1 alleles and lastly a negative control (in which DNA was replaced by H<sub>2</sub>O) was part of every sample. The PCR reaction mixtures contained the followings; PCR buffer (50mM KCl, 1.5mM MgCl<sub>2</sub>, 10mM Tris- Hcl, pH 8.3), 0.01% w/v gelatin, 200µM of dNTP mix, 1µM of allele or group specific DRB primers<sup>13</sup>, 0.2µM of control primers (amplified the third intron of DRB1genes), 1 unit of Taq polymerase (sinagen), and 100 ng of genomic DNA. PCR amplification was under taken in a PCR set (Techne- Genius). Following initial denaturation at 95°C for 5 minutes, DNA was amplified by 30 three temperature cycles; denaturation at 95°C for 20 sec, primer annealing at 61°C for 30 sec, and extension at 72°C for 30 sec. Existence or inexistence of PCR products was detected using agarose gel electrophoresis. Following addition of 5 µl

loading buffer (40% w/v sucrose, 0.25% Bromophenol Blue), the PCR reaction mixtures were put in 2% agarose gel, and then gels underwent 15-20 minutes runs at 10 V/cm in 0.5x TBE ( 89mM Tris base, 89mM Boric acid, 2mM EDTA pH 8.0). Gels were assessed under UV illumination after being stained with ethidium bromide (1µg/ml H<sub>2</sub>O) for 15 min.

The frequencies of HLA alleles were obtained by direct counting. HLA frequencies in SLE patients, control population, and lupus patients with and without specific clinical manifestations were compared, using Chi-square test or Fisher's exact probability test. A P value< 0.05 was considered to be statistically significant. SPSS version 20 software was used for analyses.

### Results

The mean age of the population studied was 30.7±10.8 years. Gene frequencies of the HLA-DRB1, DRB3, DRB4, DRB5, and DQB1 alleles in patients and controls are shown in Table 1.

**Table (1) Gene Frequencies of the HLA-DR Alleles in Controls and Patients**

Gene	Allele	Control (n = 30)		SLE Patients (51)		OR	P
		n	%	n	%		
DRB1*	01	1	3.33	1	1.96	0.58	>0.999
	03	10	33.3	18	35.3	1.09	0.857
	04	3	10	15	29.4	3.75	0.072
	07	18	60	20	39.2	0.43	0.070
	08	0		0	Undefined	Undefined	Undefined
	09	0		0	Undefined	Undefined	Undefined
	10	3	10	5	9.8	0.97	>0.999
	11	3	10	6	11.8	0.97	>0.999
	12	1	3.33	0	Undefined	0.0	0.740
	13	9	30	10	19.6	0.56	0.287

	14	0		1	1.96	Undefined	>0.999
	15	6	20	19	37.3	2.37	0.104
	16	1	3.33	1	1.96	0.58	>0.999
DRB3		21	70	24	47.1	0.38	0.044
DRB4		19	63.3	32	62.7	0.97	0.957
DRB5		7	23.3	18	35.3	1.79	0.261
DQB1*02		20	66.7	30	58.8	0.71	0.483
DQB1*03		8	26.7	22	43.1	2.08	0.138
DQB1*04		0		2	3.9	Undefined	0.787
DQB1*05		7	23.3	8	15.7	0.61	0.392
DQB1*06		16	53.3	20	32.2	0.56	0.217

The most frequent DRB1 alleles found in the SLE group were HLA-DRB1 \* 07, DRB1 \* 015, DRB1 \*3, and DRB1\* 04. However, only the HLA DRB3 was significantly decreased in SLE patients (study group) when compared to controls (p value = 0.044), whereas the frequency of DRB4, DQB1\*02, DRB3 were increased with non-significant difference when compared to controls, Only DRB3 (p value=0.044) was significantly decreased when compared to controls. It was found that HLA DRB3 frequency was increased in SLE patients with serositis, the number of which was 16 cases in comparison to those without serositis with (p value = 0.004), increased frequency of HLA DQB1\*3 with p value (0.041) among SLE patients with skin manifestations, the number of which was 37 cases versus those without skin manifestations, an increased HLA DRB1\*15 (p value 0.029) among SLE patients with nephritis, the number of which was 43 cases versus those without

nephritis, whereas HLA DRB1\*10 was found to be increased among SLE patients with hematological manifestations the number of which was 37 cases versus those without hematological manifestations, (p value =0.03), moreover HLA DRB1\*11 was found to have an increased frequency among patients with central nervous system involvement (p value = 0.002) along with an increased frequency among patients with serositis( p value = 0.047). See Table 2 on comparing Saudi patients to non-Saudi patients, the most striking finding was that only 42% of the Saudi patients have DRB3, moreover this allele was found to be protective against SLE (p value= 0.019), with the relative risk among those who carry DRB3 being around 0.65 (CI= 0.45, 0.94) in comparison to controls, while all the non-Saudi patients showed the presence of 100% presence of DRB3 though it was not found to be significant.

**Table 2 Characterizations of Lupus Patients Studied**

Clinical subsets	Present		Absent		HLA allele association			P value
	<i>n</i>	%	<i>n</i>	%	HLA Allele	Present <sup>1</sup> <i>n</i>	Absent <sup>2</sup> <i>n</i>	
Arthritis	47	92.2	4	7.8	N/A			
Hematologic	37	72.6	14	27.5	DRB1*10	10	0	0.03
Serositis	16	31.4	35	68.6	DRB3	12	12	0.004
					DRB1*11	4	2	0.047
Nervous system	3	5.9	48	94.1	DRB1*11	2	4	0.002
Skin involvement	38	74.5	13	25.5	DQB1*3	19	2	0.041
Nephritis	43	30.7	138	73	DRB1*15	17	0	0.029
≥4-organ involvement	34	66.7	17	33.3	N/A			

<sup>1</sup> Patients having the particular allele and clinical manifestation

<sup>2</sup> Patients having the allele and but not the clinical manifestation

## Discussion

This study is the first of its type not only in Saudi Arabia but may be in the whole Gulf area, carried in an area where consanguineous marriages are quite common reaching about 32.4%<sup>(18)</sup> in one study and more than 57% in another.<sup>(19)</sup> The study is conducted on a Saudi population, strikingly finding a new association between HLA DRB3 and SLE, where the former was found to be protective against the latter in contradistinction to the common association that has been reported by different investigators from different ethnicities, where this association has been reported by Chai et al among Malaysian Population,<sup>(4)</sup> Smike et al also reported the same association between DRB3 and SLE among Jamaican population,<sup>(20)</sup> moreover Fraser et al referred to the same association among multiple ethnicities, though

linking its association with renal complication to the black Americans,<sup>(21)</sup> nevertheless this association is not found among the Europeans.<sup>(22)</sup>

Interestingly, we found increased frequencies in SLE patients between HLA DRB3, DRB1\*11 and serositis, skin manifestations and HLA DQB1\*3, nephritis and HLA DRB1\*15, while hematological manifestations were associated with HLA DRB1\*10 and central nervous system involvement and HLA DRB1\*11. Liphau et al found that renal involvement is associated with DRB1\*15 among a Brazilian population<sup>(23)</sup> and El Sherbini found no relation between DRB1\*15 and renal involvement,<sup>(15)</sup> while in a similar but larger scale study, Vasconcelos et al found in a Portuguese population that HLADRB1\* 08 allele frequency was increased

among SLE patients with neurological involvement.<sup>(24)</sup>

### Conclusion:

To sum up, this study highlights that, in contradistinction to what have been found among other populations DRB3 is a protective gene among Saudi's against SLE. No evidence for any role for the HLA-DRB1, DRB4, DRB5, DQB1 alleles. There was an increased HLA DRB3 frequency with serositis, DQB1\*3 skin manifestations, HLA DRB1\*15 with nephritis, DRB1\*10 with hematological manifestations and DRB1\*11 with neurological manifestations. Results from this study are to be interpreted cautiously due to the small sample size.

### Conflicts of Interest

The authors declare that they have no competing issues of interests

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