original article

Association of HLA-DRB1*15 and HLA-DQB1*06 with SLE in Saudis

Saleh Al-Motwee,[a] Dunia Jawdat,[b] Ghassan S. Jehani,[a] Hanan Anazi,[c] Abdullah Shubaili,[c] Paul Sutton,[c] Aytul F. Uyar,[d] Ali H. Hajeer[c]

From the [a]Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia; [b]Immunology, King Abdulla International Medical Research Center, Riyadh, Saudi Arabia; [c]Pathology, King Abdulaziz Medical City, Riyadh, Saudi Arabia, [d]Department of Physiology, Istanbul University, Istanbul, Turkey

Correspondence: Dr. Ali H. Hajeer · Pathology, King Abdulaziz Medical City, PO Box 22490 Riyadh, 11426 Saudi Arabia T: +966 11 801 11 11 F: +966 11 252 0130 · hajeera@ngha.med.sa

Ann Saudi Med 2013; 33(3): 229-234

DOI: 10.5144/0256-4947.2013.229

BACKGROUND AND OBJECTIVES: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by humoral autoimmunity. The etiology of SLE is thought to be multifactorial including environmental, hormonal, and genetic factors. The human leukocyte antigen (HLA) has extensively been associated with the susceptibility to SLE; however, the association is heterogeneous among different ethnic groups. The aim of this study was to determine the association of HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 with SLE susceptibility in the Saudi population.

DESIGN AND SETTINGS: A total of 86 consecutive SLE patients attending the rheumatology clinic at King Abdulaziz Medical City, Riyadh, were recruited for this study.

METHODS: HLA types were determined by the polymerase chain reaction sequence-specific oligonucleotide (PCR-SSP) method in 86 patients and 356 control subjects.

RESULTS: The following HLA alleles were found to be positively associated with SLE: HLA-A*29 (OR=2.70; 95% CI=1.03-7.08; *P*=.0035), HLA-B*51 (OR=1.81; 95% CI=1.17-2.79; *P*=.0066), HLA-DRB1*15 (OR=1.45; 95% CI=0.98-2.29; *P*=.063), and HLA-DQB1*06 (OR=1.67; 95% CI=1.19-2.36; *P*=.0032), whereas HLA-DRB1*16 was negatively associated with the disease (OR=0.18; 95% CI=0.02-1.3; *P*=.055). HLA-DRB1*15 haplotypes were significantly associated with SLE (OR=2.01, 95% CI=1.20-3.68, *P*=.008); this was mainly due to the HLA-DRB1*15-DQB1*06 association.

CONCLUSIONS: Our data suggest an association between MHC class I and class II (HLA-A*29, HLA-B*51, HLA-DRB1*15, and HLA-DQB1*06) and susceptibility to SLE in the Saudi population. HLA-DRB1*15-DQB1*06 haplotype showed the highest risk factor for the disease that is similar to what was seen in the African American patients, suggesting shared susceptibility genetic factors among these ethnic groups.

Systemic lupus erythmatosus (SLE) is a chronic autoimmune disease characterized by inflammation of various tissues and organs of the body due to the production of autoantibodies.[1] SLE mostly affects the heart, lung, skin, joints, kidney, liver, blood vessels, and nervous system. Diagnosing SLE can be difficult as the symptoms come and go unpredictably. The disease is treatable mostly with corticosteroids and immunosuppressants, but thus far it is incurable and can be fatal. The prevalence of SLE varies among ethnicity and gender. It has been found to occur more frequently among African Americans and those of non-European descent. Also it is more common in women than in men.[2]

The etiology of SLE is unknown; however, several factors that have been considered to trigger as well as exacerbate the disease are hormones,[3] pathogens,[4] medications, and UV light.[5] Moreover, genetic factors have been strongly suggested to contribute to the disease. These were mainly shown by studies on identical twins[6] in addition to observing a higher disease frequency in the relatives of patients with SLE[7] and children of mothers with SLE.[8] Extensive studies have linked SLE susceptibility to genes of the human leukocyte antigen (HLA) region. However, the extent of this association varies among different population.

The aim of this study is to investigate the association between HLA alleles and SLE in our Saudi population.

METHODS

Patients and controls

A total of 86 consecutive SLE patients attending the rheumatology clinic at King Abdulaziz Medical City, Riyadh, were recruited for this study. All patients met at least 4 of the 11 American College of Rheumatology criteria.[9] Patients were consented and file review was conducted to collect all clinical and laboratory data. HLA results were compared with 356 ethnically matched controls.[10]

HLA typing

A total of 5 mL peripheral blood was collected in EDTA. DNA was prepared from blood leukocytes using the salting out procedure. White cells were separated using Ficoll Hypaque followed by lysis of erythrocytes in red blood cell lysis buffer and protein digestion in proteinase K solution. Finally, DNA was extracted by precipitating proteins in a saturated salt solution using the QIAamp DNA Blood Mini Kit from Qiagen (Valencia, California). All individuals were DNA typed for HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1 using polymerase chain reaction-sequence specific primer (PCR-SSP) (Deutsche Dynal AG, Hamburg, Germany) using low-resolution typing method.

Statistical analysis

The maximum likelihood estimates of allele frequencies and haplotype frequencies were computed using an expectation maximization algorithm by the Arlequin software.[11] To compare the differences between the allele frequencies in the controls and SLE groups, a 2×2 contingency table analysis was performed using the Pearson chi-square tests with Fisher exact test, when the expected value for an HLA marker was <5. The strength of association between HLA alleles and SLE was estimated by odds ratios (OR) and 95% confidence intervals (95% CI). $P<.05$ was considered to be statistically significant. For the 2-locus haplotypes, the the standardized disequilibrium coefficient (D') and the chi-square values were also calculated.

Table 1. Demographics of the SLE patients.

Characteristics	
Total patient number	86
F:M	10.7:1
Age range of patients	9-60 y
Mean age at onset (SD)	26.1 10.2 y
Disease duration mean (SD)	8.8 (5) y

SLE: Systemic lupus erythematosus, F:M: female to male ratio, SD: standard deviation.

RESULTS

We investigated HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 genes in 86 Saudi SLE patients and compared their results with 356 healthy controls. The female gender dominated showing a ratio of female to male as 10.7:1, with the mean age of 26.1 years at onset and the mean disease duration of 8.8 years (**Table 1**). Arthritis was the most common clinical presentation, followed by renal involvement, malar

Table 2. Clinical presentation of SLE patients.

Clinical presentation	No. (%)
Arthritis	71 (82.6)
Renal involvement	30 (34.9)
Alopecia	19 (22.1)
Raynaud phenomenon	7 (8.1)
Photosensitivity	11 (12.8)
Hemolytic anemia	13 (15.1)
CNS involvement	4 (4.7)
Malar rash	25 (29.1)
Oral ulcers	13 (15.1)
Leukopenia	25 (29.1)
Thrombocytopenia	9 (10.5)
Serositis	4 (4.7)
Pleuritis	4 (4.7)

SLE: Systemic lupus erythematosus, CNS: central nervous system.

Table 3. Number and percentage of autoantibody positive SLE patients.

Autoantibody	No. (%)
ANA	85 (98.9)
Anti-DNA	85 (98.9)
Low C3 or C4	80 (93.0)
RNP antibodies	12 (14.0)
Anti-Sm	5 (5.8)
Anti-cardiolipin	39 (45.3)
Anti-Ro	23 (26.7)
Anti-La	10 (11.6)

SLE: Systemic lupus erythematosus.

rash, leucopenia, and alopecia (**Table 2**). Most patients presented with ANA (98.9%), anti-DNA (98.9%), and low complement C3 and C4 (93.0%), (**Table 3**).

Tables 4 to 7 show the HLA class I and class II allele frequencies in both SLE cases and controls. The following HLA types were significantly increased in cases versus controls: HLA-A*29 (OR 2.70, 95% CI 1.03-7.08, *P*=.035) and HLA-DQB1*06 (OR 1.67, 95% CI 1.19-2.36, *P*=.032). However, HLA-B*51 (OR 1.81, 95% CI 1.17-2.79, *P*=.0066) and HLA-DRB1*15 (OR 1.49, 95% CI 0.98-2.29, *P*=.063) were marginally significant. HLA-DRB1*01 (OR 0.16, 95% CI 0.02-1.2, *P*=.041) was protective, whereas HLA-A*02 (OR 0.69, 95% CI 0.47-1.02, *P*=.06) and HLA-DRB1*16 (OR 0.18, 95% CI 0.02-1.3, *P*=.055) were marginally protective.

Table 8 describes the association between HLA-DRB1*15 haplotypes and SLE. Apparently, HLA-DRB1*15-DQB1*06 haplotype carried a significant risk for SLE (OR 2.01, 95% CI 1.20-3.68, *P*=.008) in our Saudi population.

DISCUSSION

We investigated the association of HLA genes in a Saudi cohort of SLE patients. This is the first description of immunogenetics of SLE in Saudi Arabia. The age at onset and the preponderance of females over males in this cohort were similar to other populations.[12]

Two major HLA haplotypes have been shown repeatedly to be associated with SLE worldwide: HLA-DR3 and HLA-DR2 (DR15 and DR16) bearing haplotypes.[13-16] Different HLA-DR alleles were reported in different ethnics groups: HLA-DRB1*0301 with Caucasians, HLA-DRB1*1503 with African Americans, and HLA-DRB1*08 alleles with Hispanics. In our population, HLA-DRB1*15 haplotypes were found to be associated with SLE in Saudis, while HLA-DRB1*16 was protective. In Mexicans, DR15 haplotypes were found to be associated with risk for SLE,[17] while, 1 study showed that HLA-DRB1*16 was associated with chronic discoid lupus in Mexicans.[18] HLA-DRB1*04 was protective

Table 4. HLA-A associations with SLE in Saudi patients.

SLE	Controls				OR	95% CI	*P*
	N	Frequency	N	Frequency			
HLA-A*01	17	0.099	51	0.072			
HLA-A*02	40	0.233	217	0.305	0.69	0.47-1.02	.06
HLA-A*03	7	0.041	46	0.065			
HLA-A*11	8	0.047	27	0.038			
HLA-A*23	10	0.058	38	0.053			
HLA-A*24	16	0.093	53	0.074			
HLA-A*25	0	0.000	1	0.001			
HLA-A*26	11	0.058	33	0.046			
HLA-A*29	7	0.041	11	0.015	2.70	1.03-7.08	.035
HLA-A*30	7	0.041	39	0.055			
HLA-A*31	16	0.093	50	0.070			
HLA-A*32	4	0.023	37	0.052			
HLA-A*33	7	0.041	43	0.060			
HLA-A34	2	0.012	0	0.003			
HLA-A66	1	0.006	1	0.001			
HLA-A68	15	0.081	55	0.077			
HLA-A69	2	0.012	0	0.000			
HLA-A74	2	0.012	8	0.011			

SLE: Systemic lupus erythematosus, OR: odds ratio, HLA: human leukocyte antigen.

Table 5. HLA-B associations with SLE in Saudi patients.

SLE	Controls				OR	95% CI	*P*
	N	Frequency	N	Frequency			
HLA-B*07	20	0.116	69	0.097			
HLA-B*08	13	0.076	57	0.08			
HLA-B*13	1	0.006	10	0.014			
HLA-B*14	1	0.006	7	0.01			
HLA-B*15	7	0.041	32	0.045			
HLA-B*18	1	0.006	22	0.031			
HLA-B*27	0	0	6	0.008			
HLA-B*35	14	0.081	58	0.081			
HLA-B*37	2	0.012	8	0.011			
HLA-B*38	4	0.023	9	0.013			
HLA-B*39	5	0.029	6	0.008			
HLA-B*40	6	0.035	11	0.015			
HLA-B*41	9	0.052	28	0.039			
HLA-B*42	3	0.017	8	0.011			
HLA-B*44	4	0.023	26	0.037			
HLA-B*45	0	0	2	0.003			
HLA-B*46	0	0	2	0.003			
HLA-B*47	0	0	1	0.001			
HLA-B*49	1	0.006	27	0.038			
HLA-B*50	23	0.134	137	0.192			
HLA-B*51	35	0.203	88	0.124	1.81	1.17-2.79	.0066
HLA-B*52	3	0.017	11	0.015			
HLA-B*53	7	0.041	31	0.044			
HLA-B*54	0	0	1	0.001			
HLA-B*55	2	0.012	5	0.007			
HLA-B*56	3	0.017	0	0			
HLA-B*57	2	0.012	15	0.021			
HLA-B*58	6	0.035	27	0.038			
HLA-B*67	0	0	2	0.003			
HLA-B*73	0	0	5	0.007			
HLA-B*78	0	0	1	0.001			

SLE: Systemic lupus erythematosus, OR: odds ratio, HLA: human leukocyte antigen.

Table 6. HLA-DRB1 associations with SLE in Saudi patients.

SLE		Controls			OR	95% CI	*P*
	N	Frequency	N	Frequency			
HLA-DRB1*01	1	0.006	25	0.035	0.16	0.02-1.20	.041
HLA-DRB1*15	35	0.203	104	0.146	1.49	0.98-2.29	.063
HLA-DRB1*16	1	0.006	23	0.032	0.18	0.02-1.30	.055
HLA-DRB1*03	30	0.174	103	0.145			
HLA-DRB1*04	17	0.099	114	0.16			
HLA-DRB1*11	12	0.07	49	0.069			
HLA-DRB1*12	0	0	5	0.007			
HLA-DRB1*13	29	0.169	94	0.132			
HLA-DRB1*14	1	0.006	7	0.01			
HLA-DRB1*07	33	0.192	144	0.202			
HLA-DRB1*08	5	0.029	7	0.01			
HLA-DRB1*09	1	0.006	0	0			
HLA-DRB1*10	7	0.041	37	0.052			

SLE: Systemic lupus erythematosus, OR: odds ratio, HLA: human leukocyte antigen.

Table 7. HLA-DQB1 associations with SLE in Saudi patients.

SLE		Controls			OR	95% CI	*P*
	N	Frequency	N	Frequency			
HLA-DQB1*02	60	0.349	245	0.344			
HLA-DQB1*03	34	0.198	164	0.23			
HLA-DQB1*04	3	0.017	19	0.027			
HLA-DQB1*05	5	0.029	77	0.108			
HLA-DQB1*06	70	0.407	207	0.291	1.67	1.19-2.36	.0032

SLE: Systemic lupus erythematosus; OR: odds ratio, HLA: human leukocyte antigen.

in this Saudi population; the same result was observed in patients from Northwest Spain.[19]

Several studies analyzed the MHC region for genetic risk of SLE. Graham et al[20] narrowed the disease-associated haplotypes HLA-DRB1*1501-HLA-DQB1*0602 and HLA-DRB1*0801-HLA-DQB1*0402 to a region of 500 kb. Fernando et al[21] using British SLE families and TdT analysis, narrowed the susceptibility region in MHC to 180 kb that involved the HLA-DRB1*0301-HLA-DQA1*0501-HLA-DQB1*0201. Our own results suggested that HLA-DRB1*15-HLA-DQB1*06 haplotype is a risk factor for SLE in Saudis; however, looking at the allele frequencies we find that the frequency of HLA-DRB1*15 is nearly 20% whereas that of HLA-DQB1*06 is 40%, suggesting that HLA-DQB1*06 is associated with SLE independent of HLA-DRB1*15. Thus narrowing the risk area of SLE to the DQB1 region, it still remains elusive whether HLA-DQB1*06 is the culprit or another gene polymorphism is in linkage disequilibrium with it.

One third of our patients have renal involvement; whereas, in other Asian populations, renal involvement ranged from 18% to 100%, majority reporting >50% of their patients.[22] In Italians, lupus nephritis was found to be associated with the HLA-DR15-bearing haplotypes;[23] this was also reported in other studies.[24-26] In our patients, there was no association between HLA-DR15-bearing haplotypes and lupus nephritis (data not shown). Alarcón et al[27] analyzed factors influencing the development of lupus nephritis. Their results suggested

Table 8. Haplotypes in association with SLE.

SLE	Controls			
DRB1*15 Haplotype	N	Frequency	N	Frequency
HLA-A*02 HLA-B*07 HLA-DRB1*15 HLA-DQB1*06	8	0.047	27	.038
HLA-A*01 HLA-B*51 HLA-DRB1*15 HLA-DQB1*06	4	0.023	0	0
HLA-A*02 HLA-B*51 HLA-DRB1*15 HLA-DQB1*06	3	0.017	8	.011
HLA-A*31 HLA-B*35 HLA-DRB1*15 HLA-DQB1*06	3	0.017	6	.008
HLA-A*02 HLA-B*50 HLA-DRB1*15 HLA-DQB1*06	2	0.012	1	.001

SLE: Systemic lupus erythematosus, human leukocyte antigen.

Note: Having DRB1*15 haplotypes in SLE compared to controls OR=2.01, 95% (1.20-3.68), *P*=.008.

that younger, hypertensive, and of African American or Hispanic ethnicity were predictors of lupus nephritis risk. Moreover, end-stage renal disease was also predicted by the presence of homozygosity for the valine allele of FcγRIIIa (FCGR3A*GG).[27] This finding suggested that HLA-DR15 is not the only predictor of lupus nephritis risk and thus further analysis is required to determine the risk factors for the development of lupus nephritis in the Saudi patients.

In conclusion, this is the first study to show HLA-DRB1 and HLA-DQB1 associations with SLE in the Saudi population.

Acknowledgments

We acknowledge the support extended by King Abdullah International Medical Research Center.

REFERENCES

1. Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol 2003;56:481-490.
2. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus. 2006;15:308-18.
3. Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. Arthritis Rheum. 2007;56:1251-62.
4. James JA, Kaufman KM, Farris AD, Taylor-Albert E, Lehman TJ, Harley JB. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. J Clin Invest. 1997;100:3019-26.
5. Norris DA. Pathomechanisms of photosensitive lupus erythematosus. J Invest Dermatol. 1993;100:58S-68S.
6. Block SR, Winfield JB, Lockshin MD, D'Angelo WA, Weksler ME, Fotino M, et al. Proceedings: Twin studies in systemic lupus erythematosus (SLE). Arthritis Rheum. 1975;18:285.
7. Arnett FC, Reveille JD, Wilson RW, Provost TT, Bias WB. Systemic lupus erythematosus: current state of the genetic hypothesis. Semin Arthritis Rheum. 1984;14:24-35.
8. Murashima A, Fukazawa T, Hirashima M, Takasaki Y, Oonishi M, Niijima S, et al. Long term prognosis of children born to lupus patients. Ann Rheum Dis. 2004;63:50-3.
9. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.
10. Hajeer AH, Sawidan FA, Bohlega S, Saleh S, Sutton P, Shubaili A, et al. HLA class I and class II polymorphisms in Saudi patients with myasthenia gravis. Int J Immunogenet. 2009;36:169-72.
11. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 2005;1:47-50.
12. Gaubitz M. Epidemiology of connective tissue disorders. Rheumatology. 2006;45 Suppl 3:iii3-4.
13. Suggs MJ, Majithia V, Lewis RE, Cruse JM. HLA DRB1*1503 allelic haplotype predominance and associated immunodysregulation in systemic lupus erythematosus. Exp Mol Pathol. 2011;91:548-62.
14. Vasconcelos C, Carvalho C, Leal B, Pereira C, Bettencourt A, Costa PP, et al. HLA in Portuguese systemic lupus erythematosus patients and their relation to clinical features. Ann N Y Acad Sci. 2009;1173:575-80.
15. Graham RR, Ortmann W, Rodine P, Espe K, Langefeld C, Lange E, et al. Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE. Eur J Hum Genet. 2007;15:823-30.
16. Reveille JD, Schrohenloher RE, Acton RT, Barger BO. DNA analysis of HLA-DR and DQ genes in American blacks with systemic lupus erythematosus. Arthritis Rheum. 1989;32:1243-51.
17. Cortes LM, Baltazar LM, Lopez-Cardona MG, Olivares N, Ramos C, Salazar M, et al. HLA class II haplotypes in Mexican systemic lupus erythematosus patients. Hum Immunol. 2004;65:1469-76.
18. López-Tello A, Rodríguez-Carreón AA, Jurado F, Yamamoto-Furusho JK, Castillo-Vázquez M, Chávez-Muñoz C, et al. Association of HLA-DRB1*16 with chronic discoid lupus erythematosus in Mexican mestizo patients. Clin Exp Dermatol. 2007;32:435-8.
19. al-Ansari AS, Hajeer AH, Garcia-Porrua C, Thomson W, Ollier WE, Gonzalez-Gay MA. HLA-DRB1 associations in systemic lupus erythematosus patients from northwest Spain. Clin Exp Rheumatol. 2001;19:352.
20. Graham RR, Ortmann WA, Langefeld CD, Jawaheer D, Selby SA, Rodine PR, et al. Visualizing human leukocyte antigen class II risk haplotypes in human systemic lupus erythematosus. Am J Hum Genet. 2002;71:543-53. Epub 2002 Jul 26.
21. Fernando MM, Stevens CR, Sabeti PC, Walsh EC, McWhinnie AJ, Shah A, et al. Identification of two independent risk factors for lupus within the MHC in United Kingdom families. PLoS Genet. 2007;3:e192.
22. Osio-Salido E, Manapat-Reyes H. Epidemiology of systemic lupus erythematosus in Asia. Lupus. 2010;19:1365-73.
23. Marchini M, Antonioli R, Lleò A, Barili M, Caronni M, Origgi L, et al. HLA class II antigens associated with lupus nephritis in Italian SLE patients. Hum Immunol. 2003;64:462-8.
24. Arnett FC, Reveille JD. Genetics of systemic lupus erythematosus. Rheum Dis Clin North Am. 1992;18:865-92.
25. Doherty DG, Ireland R, Demaine AG, Wang F, Veerapan K, Welsh KI, et al. Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in southern Chinese. Arthritis Rheum. 1992;35:641-6.
26. Fronek Z, Timmerman LA, Alper CA, Hahn BH, Kalunian K, Peterlin BM, et al. Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. Arthritis Rheum. 1990;33:1542-53.
27. Alarcón GS, McGwin G Jr, Petri M, Ramsey-Goldman R, Fessler BJ, Vilá LM, et al. Time to renal disease and end-stage renal disease in PROFILE: a multiethnic lupus cohort. PLoS Med. 2006;3:e396.