Systemic lupus erythematous (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.

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.
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. Patients were consented and file review was conducted to collect all clinical and laboratory data. HLA results were compared with 356 ethnically matched controls.

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. 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 test 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.

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...
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.

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

| HLA-A | SLE | Controls | N | Frequency | N | Frequency | OR | 95% CI | P  |
|-------|-----|----------|---|-----------|---|-----------|----|-------|----|
| A*01  |    |          | 17 | 0.099     | 51 | 0.072     |    |        |    |
| A*02  |    |          | 40 | 0.233     | 217| 0.305     | 0.69| 0.47-1.02 | .06|
| A*03  |    |          | 7  | 0.041     | 46 | 0.065     |    |        |    |
| A*11  |    |          | 8  | 0.047     | 27 | 0.038     |    |        |    |
| A*23  |    |          | 10 | 0.058     | 38 | 0.053     |    |        |    |
| A*24  |    |          | 16 | 0.093     | 53 | 0.074     |    |        |    |
| A*25  |    |          | 0  | 0.000     | 1  | 0.001     |    |        |    |
| A*26  |    |          | 11 | 0.058     | 33 | 0.046     |    |        |    |
| A*29  |    |          | 7  | 0.041     | 11 | 0.015     | 2.70| 1.03-7.08 | .035|
| A*30  |    |          | 7  | 0.041     | 39 | 0.055     |    |        |    |
| A*31  |    |          | 16 | 0.093     | 50 | 0.070     |    |        |    |
| A*32  |    |          | 4  | 0.023     | 37 | 0.052     |    |        |    |
| A*33  |    |          | 7  | 0.041     | 43 | 0.060     |    |        |    |
| A*34  |    |          | 2  | 0.012     | 0  | 0.003     |    |        |    |
| A*66  |    |          | 1  | 0.006     | 1  | 0.001     |    |        |    |
| A*68  |    |          | 15 | 0.081     | 55 | 0.077     |    |        |    |
| A*69  |    |          | 2  | 0.012     | 0  | 0.000     |    |        |    |
| A*74  |    |          | 2  | 0.012     | 8  | 0.011     |    |        |    |

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

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. 

Two major HLA haplotypes have been shown repeatedly to be associated with SLE worldwide: HLA-DR3 and HLA-DR2 (DR15 and DR16) bearing haplotypes. Different HLA-DR alleles were reported in different ethnic 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, while 1 study showed that HLA-DRB1*16 was associated with chronic discoid lupus in Mexicans. HLA-DRB1*04 was protective.
### 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  |
| 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     |
|-----------|----------|-----|------------|-------|
| HLA-DRB1*01 | 1        | 0.035 | 0.16      | 0.02-1.20 | .041 |
| HLA-DRB1*15 | 35       | 0.146 | 1.49      | 0.98-2.29 | .063 |
| HLA-DRB1*16 | 1        | 0.032 | 0.18      | 0.02-1.30 | .055 |
| HLA-DRB1*03 | 30       | 0.145 | 1.6       |          |      |
| HLA-DRB1*04 | 17       | 0.089 |           |        |      |
| HLA-DRB1*11 | 12       | 0.07  | 0.069     |        |      |
| HLA-DRB1*12 | 0        | 0     | 0.007     |        |      |
| HLA-DRB1*13 | 29       | 0.132 |           |        |      |
| HLA-DRB1*14 | 1        | 0.01  |           |        |      |
| HLA-DRB1*07 | 33       | 0.202 |           |        |      |
| HLA-DRB1*08 | 5        | 0.01  |           |        |      |
| HLA-DRB1*09 | 1        | 0     | 0         |        |      |
| HLA-DRB1*10 | 7        | 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     |
|-----------|----------|-----|------------|-------|
| HLA-DQB1*02 | 60       | 0.344 |           |       |
| HLA-DQB1*03 | 34       | 0.23  |           |       |
| HLA-DQB1*04 | 3        | 0.027 |           |       |
| HLA-DQB1*05 | 5        | 0.108 |           |       |
| HLA-DQB1*06 | 70       | 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 al20 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 al21 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 al27 analyzed factors influencing the development of lupus nephritis. Their results suggested
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 FcRIIIa (FCGR3A*GG). 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;15:201-18.
3. Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. Arthritis Rheum. 2007;56:1211-2.
4. James JA, Kaufman KM, Farriss 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. 1993;100:58S-68S.
7. 1997;100:3019-26.
8. 2003;56:481-490.
9. 2006;15:308-18.
10. 1975;18:285.
11. 1997;10:457-450.
12. 2000:4:157-62.
13. 2004:3:363-4.
14. 2001;19:352.
15. 2007:15:823-30.
16. 2007:15:823-30.
17. 2005;1:47-50.
18. 2001;19:352.
19. 2006;45 Suppl 3:i3-4.
20. 2002;32:1243-51.
21. 2009;1173:575-80.
22. 2003;32:1243-51.
23. 2007:3:18-26.
24. 2011;547-60.
25. 2003;1:47-50.
26. 2001:19:352.
27. 2009;36:169-72.
28. 2009;3:363-4.
29. 2007:15:823-30.
30. 2003;56:481-490.
31. 2006;32:435-8.
32. 2007:3:18-26.
33. 2001;19:352.
34. 2009:1:47-50.
35. 2005;1:47-50.
36. 2001:19:352.
37. 2009;1173:575-80.
38. 2006;32:435-8.
39. 2007:3:18-26.
40. 2007:3:18-26.
41. 2001:19:352.
42. 2009:1173:575-80.
43. 2007:3:18-26.
44. 2001:19:352.
45. 2009:1173:575-80.
46. 2007:3:18-26.
47. 2009:1173:575-80.