

The involvement of HLA - DRB1*, DQA1*, DQB1* and complement C4A Loci in diagnosing systemic lupus erythematosus among Tunisians

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Background: Genetic susceptibility to systemic lupus erythematosus (SLE) varies among populations. Few data exist on associations of HLA class II and class III alleles of the major histocompatibility complex (MHC) and susceptibility to SLE in Tunisians.

Patients and Methods: We compared HLA-DRB1*, DQA1, DQB1* and C4 allotypes in 62 Tunisian SLE patients and 100 matched controls. We also assessed the association of specific alleles with distinct autoantibody profiles in SLE patients.

Results: HLA-DRB1*0301, -DRB1*1501 and C4AQO alleles were increased in the SLE patients, while the frequencies of HLA-DRB1*04 and DQB1*03 were decreased. HLA-DQA1*0102 and DQA1*0501 were significantly increased in the SLE patients. HLA-DQB1*0201 and DQB1*0602 were more frequent in the SLE patients. C4A*QO and C4B*QO were increased in frequency in the SLE patients compared to the controls, but only C4A null was significantly increased. Eleven of 17 SLE patients with the C4 null allele were HLA-DRB1*0301 positive. Three of 16 SLE patients with HLA-DRB1*1501 were associated with HLA-DQB1*0501 rather than DQB1*0602, as has been reported in European SLE patients.

Conclusions: The MHC class II alleles (DRB1, DQA1, DQB1) and C4 null associations noted in other ethnic groups are also found in Tunisians, suggesting shared susceptibility factors across ethnic lines in predisposition to SLE. In contrast to other ethnic groups, MHC class II alleles are not associated with the presence of specific autoantibodies in Tunisian SLE patients.

Key words: Systemic lupus erythematosus, major histocompatibility complex, HLA antigens, complement C4A, autoantibodies, Tunisia

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease of unknown aetiology with a high incidence among females, particularly during childbearing years.^{1,30} Susceptibility factors include environmental exposures such as UV light and drugs and, in genetically predisposed individuals, viral infections.² The genes involved in SLE have not yet been identified with certainty, but most studies implicate genes within the major histocompatibility complex (MHC).³ There are multiple studies of the HLA-DR and DQ alleles in SLE patients in different ethnic populations. In white patients with SLE the most consistent findings have been increases in HLA-DR2, DR3 or in both. More recent studies using DNA typing have identified disease-associated haplotypes and have extended these initial associations to other racial and ethnic groups. The HLA-DR2 association with SLE is accounted for by the HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotype, which has been reported in Central European Caucasians,³ Chinese,⁴ Japanese,⁵ and African-Americans.^{6,7} The HLA-DR3 haplotype bearing HLA-DRB1*0301-DQA1*0501-DQB1*0201 was observed

in Central and Western Europeans, and North Americans.⁷⁻⁹ However, in the majority of other ethnic groups studied, no MHC class II associations have been found.¹⁰⁻¹²

Inherited complement deficiencies also predispose to SLE. Thus, C4A null alleles or gene deletions have been associated with SLE in Caucasians¹³⁻¹⁵ South Africans,¹⁶ Japanese^{5, 25} Koreans¹⁷ and African-Americans.¹⁸ Linkage disequilibrium of HLA-DRB1*1501 and DRB1*0301 with specific DQA1, DQB1 and C4 genes has complicated determinations of which loci were more important in disease predisposition. To better address this, HLA associations must be examined in other ethnic groups where linkage disequilibrium among HLA-DR, DQ and C4 alleles may not be so strong. More recently, it has been determined that class II alleles show stronger associations with the presence of specific autoantibodies than with SLE itself. These autoantibody subsets are themselves associated with specific clinical manifestations. Therefore, genetic factors are likely to play a determining role in the appearance of SLE.^{9, 14, 19}

Tunisians comprise a group of largely Arabic and Berber genetic admixture. It is impossible to distinguish between

the two origins. There are few data on HLA alleles and predisposition to SLE in this ethnic group. We examined HLA-DRB1, DQA1, DQB1 and C4 alleles in Tunisian SLE patients and matched local controls. In addition, we assessed the association of specific alleles with distinct autoantibody profiles in SLE patients.

Patients and Methods

Sixty-two Tunisian patients with SLE (57 females [92%], 5 males 8%) attending Charles Nicolle Hospital, Tunis, met four or more revised criteria of the American College of Rheumatology (ACR) for the classification of SLE.²⁰ Healthy control subjects were 100 Tunisian individuals selected at random.

Peripheral blood was collected on EDTA. Genomic DNA was isolated using a previously published salting-out method or by phenol/chloroform extraction. HLA class II genes were typed and subtyped by a polymerase chain reaction/sequence-specific primer (PCR/SSP) method. Patients and controls were typed for HLA-DRB1, DQA1, and DQB1 alleles by PCR/SSP Dynal kits (Dynal, France) following the manufacturer recommendations. C4 allotyped and C4 null alleles were determined using carboxypeptidase and neuraminidase-treated plasma, run on agarose gel electrophoresis and subjected to immunofixation.²⁷ Allotype assignment was carried out as recommended by the XI International Complement Genetics Workshop²¹ Anti-Ro (SS-A), La (SS-B), Sm and RNP antibodies were determined by Elisa assays (Sanofi-Pasteur, France). Anti-double-stranded (ds) DNA antibodies were determined by indirect immunofluorescence against *Crithidia luciliae*.

Frequencies of DRB1, DQA1 and DQB1 alleles in the 62 patients with SLE were compared with those in 100 healthy unrelated Tunisian controls. Relative risk (as calculated by Woolf²⁸) and the statistical significance of the association between SLE and genetic markers were assessed by the chi-square test with Yates' correction. A corrected *P* value was calculated by multiplying the *P* value by the number of alleles at the respective locus. Linkage disequilibrium was tested by the method of Mittal.²⁹

Results

In 62 patients examined for autoantibodies, anti-ds DNA was found in 55 patients (88.7%), anti-Ro (SSA)/anti-La (SSB) in 20 patients (32.2%), anti-Sm in 21 patients (38.8%), and anti-U1 RNP in 17 patients (31.4%). A positive association was seen between HLA-DRB alleles and SLE for DRB1*0301 and DRB1*1501, while a negative association was seen for DRB1*11 (Table 1). DQA1*0102 and DQA1*0501 were slightly more frequent in patients compared to controls, probably due to linkage disequilibrium with DRB1*1501 and DRB1*0301 in the Tunisian population compared with other ethnic

groups whereas DQB1*0201 and DQB1*0602 were more frequent in patients compared to controls (Table 2). DRB1*1501 was in linkage disequilibrium with the DQA1*0102 and DQB1*0602 and DRB1*0301 was in disequilibrium with DQA1*0501 and DQB1*0201 haplotypes in Tunisians. DRB1-DQA1-DQB1 haplotypes were deduced in the patients and controls (Table 3). Frequencies of the DRB1*1501-DQA1*0102-DQB1*0602 and DRB1*0301-DQA1*0501-DQB1 0201 haplotypes were significantly increased. Six patients (9.6%) and two controls had the combination of two haplotypes. No specific HLA class II haplotypes or alleles were significantly associated with any of the specific antibodies.

Forty-one of the SLE patients and 169 local Tunisian controls²⁷ had sera available for C4 allotyping. Twenty-one of these SLE patients had no measurable C4, possibly due to active SLE and complement degradation during shipping. Both C4A*QO and C4B*QO were increased in frequency in the SLE patients compared to the controls, but only C4AQO was significant (20.7% vs. 8.9% corrected *P*<0.002, RR =7.11). Eleven of seventeen SLE patients with a C4 null allele were HLA-DRB1*0301 positive. There were no homozygotes for C4A*QO among the SLE patients and there was no association of the C4A*QO phenotypes with the presence of specific autoantibodies.

Discussion

SLE has a worldwide distribution, a predilection for young females and a heterogeneous clinical expression.²² HLA region genes have been implicated in susceptibility to the disease.²⁴ In Caucasians, the association is mainly with DR3 (DRB1*0301) and DR2 (DRB1*1501) or both. However, in black Americans associations have been variously described with DR3, both DR2 and DR3 and DR7, though these have not been confirmed in other studies. The present study was conducted to determine the associations of MHC class II alleles and the prevalence of C4 deficiencies in a cohort of 62 patients with SLE. Our results confirm that SLE in Tunisians is associated with DRB1*0301 and its linked alleles (DQA1*0501-DQB1*0201 haplotype), through linkage disequilibrium. The C4 null phenotype (especially a C4A null allotype) was also significantly increased in SLE patients (due to linkage disequilibrium with DR3) as has been observed in other racial and ethnic groups.^{13,14} However, a disassociation of these two risk factors (DR3 and C4 null phenotype) has been observed in Spanish and Mexican SLE patients.^{18,21} HLA-DRB1*1501 and its linked alleles (DQA1*0102-DQB1*0602) were also increased.

Apart from reaffirming the association of SLE to HLA-DR-DQ, our findings are consistent with the presence of an extended haplotype in Tunisian SLE patients. The finding that DRB1*1501 shows a strong allelic association to HLA-

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Table 1. Frequency of HLA-DRB1 alleles in patients with systemic lupus erythematosus (SLE) and healthy controls.

| DRB1* | SLE (n=62) | | Controls (n=100) | | Statistical comparison of phenotypes | |
|-------|---------------|-------------|------------------|-------------|--------------------------------------|---------------|
| | Phenotype (%) | Allelic (%) | Phenotype (%) | Allelic (%) | P value* | Relative risk |
| O1 | 11.29 | 5.9 | 16 | 8.4 | 0.002 | 3.76 |
| O301 | 50 | 29.3 | 23 | 12.3 | | |
| O4 | 17.74 | 9.3 | 26 | 14 | | |
| 7 | 30.64 | 16.7 | 31 | 17 | 0.04 | 0.4 |
| 11 | 12.9 | 6.7 | 27 | 14.6 | | |
| 12 | 0 | 0 | 7 | 3.6 | | |
| 9 | 0 | 0 | 1 | 0.6 | 0.001 | 2.94 |
| 13 | 17.74 | 9.3 | 27 | 14.6 | | |
| 14 | 1.61 | 0.9 | 5 | 2.6 | | |
| 1501 | 32.25 | 17.7 | 13 | 6.8 | 0.001 | 2.94 |
| 16 | 4.83 | 2.5 | 2 | 1.11 | | |
| 8 | 4.83 | 2.5 | 5 | 2.6 | | |
| 10 | 4.83 | 2.5 | 2 | 1.2 | | |

* Corrected

Table 2. Frequency of HLA-DQA1 and DQB1 alleles in patients with systemic lupus erythematosus (SLE) and healthy controls.

| | SLE (n=62) | | Controls (n=92) | | Statistical comparison of phenotypes | |
|--------------|---------------|-------------|-----------------|-------------|--------------------------------------|---------------|
| | Phenotype (%) | Allelic (%) | Phenotype (%) | Allelic (%) | P value* | Relative risk |
| DQA1* | | | | | | |
| 0101 | 11.86 | 62 | 18.47 | 9.8 | 0.007 | 3.09 |
| 0102 | 45.16 | 25.94 | 22.82 | 12.2 | | |
| 0103 | 5.08 | 2.6 | 8.69 | 4.5 | | |
| 0104 | 1.7 | 0.9 | 3.26 | 1.7 | 0.03 | 4.3 |
| 0105 | 5.08 | 2.6 | 6.52 | 3.3 | | |
| 0201 | 28.81 | 15.7 | 31.52 | 17.24 | | |
| 0301 | 8.47 | 4.4 | 19.56 | 10.31 | 0.003 | 4.3 |
| 0302 | - | - | 2.17 | 1.09 | | |
| 0303 | 11.86 | 62 | 4.34 | 2.19 | | |
| 0401 | 1.7 | 0.9 | 3.26 | 1.7 | 0.003 | 4.3 |
| 0501 | 50.84 | 29.9 | 31.52 | 17.24 | | |
| 0505 | 13.56 | 7.1 | 27.17 | 14.65 | | |
| 0601 | 1.7 | 0.9 | 1.08 | 0.54 | 0.003 | 4.3 |
| Blank | 15.25 | - | 20.25 | - | | |
| DQB1* | | | | | | |
| 0201 | 46.77 | 27 | 17 | 8.9 | 0.003 | 4.3 |
| 0202 | 25.80 | 13.9 | 32 | 15.8 | | |
| 0301 | 20.96 | 11.1 | 38 | 21.3 | | |
| 0302 | 9.67 | 5 | 19 | 10 | 0.003 | 4.3 |
| 0303 | 11.29 | 5.9 | 5 | 2.6 | | |
| 0305 | 1.61 | 0.9 | 4 | 2.1 | | |
| 0402 | 3.22 | 1.7 | 8 | 4.1 | 0.003 | 4.3 |
| 0501 | 20.96 | 11.1 | 23 | 12.3 | | |
| 0502 | 9.67 | 5 | 6 | 3.1 | | |
| 0503 | 1.61 | 0.9 | 5 | 2.6 | 0.003 | 4.3 |
| 0601 | 3.22 | 1.7 | 6 | 3.1 | | |
| 0602 | 29.03 | 15.8 | 6 | 3.1 | | |
| 0603 | 1.61 | 0.9 | 5 | 2.6 | 0.003 | 4.3 |
| 0604 | 6.45 | 3.3 | 6 | 3.1 | | |
| 0609 | - | - | 6 | 3.1 | | |
| Blank | 8.06 | 4.1 | 14 | - | | |

* Corrected

Table 3. Frequency of HLA-DR, DQA, DQB haplotypes in patients with systemic lupus erythematosus (SLE) and healthy controls.

| HLA haplotypes | SLE | | Controls | | Delta max | Corrected P value | Relative risk |
|---|-----|-------|----------|------|-----------|-------------------|---------------|
| | n | HF | n | HF | | | |
| DRB1*1501-DQA1*0102-DQB1*0602 | 18 | 0.352 | 4 | 0.04 | 0.278 | 0.001 | 9.4 |
| DRB1*0301-DQA1*0501-DQB1*0201 | 29 | 0.467 | 16 | 0.16 | 0.3812 | 0.001 | 10.10 |
| DRB1*1501-0301,DQA1*0102-0501, DQB1*0201-0602 | 6 | 0.096 | 2 | 0.02 | - | 0.004 | 1.8 |

HF=haplotype frequency

DQA1*0102-DQB1*0602 in SLE compared to controls suggests that the linkage is being selectively maintained in lupus. A similar increase was noted in African-American SLE patients.¹³

Some of the observed associations of SLE with specific genetic markers in the MHC are likely due to founder effects, whereby the original SLE mutation arose on a chromosome carrying those markers, although the markers themselves are not responsible for causing the condition. Founder effects may explain the variety of HLA-DR alleles that appear to be linked to the disease in different populations. The association of DRB1*0301 with DQB1*0201, DQA1*0501 and DRB1*1501 with DQB1*0602, DQA1*0102 indicates the presence of at least two founders in this Tunisian population. The gene(s) controlling susceptibility to SLE may be one, or the combination of two or more of HLA DRB1*0301-DQA1*0501- DQB1*0201 or DRB1*01501-DQA1*0102 -DQB1*0602 or an unidentified gene in strong linkage disequilibrium with the DRB1*0301 and DRB1*1501 haplotypes.²⁶

It now appears likely that partial deficiencies of C4, especially of the C4A isotype, are independent risk factors for SLE on linked MHC class II alleles.²³ The basis for the association of the C4 null allotype with SLE is unclear.

The C4A isotype is known to trans-acetylate with proteins more efficiently than C4B does. C4A is more efficient in its interaction with immune complexes and may be better able to participate in complement-mediated processing and clearance of immune complexes. Thus, patients deficient in C4A lack the isotype of C4, which may be most important in handling immune complexes. C4AQO is frequent in SLE in different ethnic groups irrespective of the HLA type, and seems to cross ethnic barriers.^{4, 18, 23} However, recent studies indicate that the genetic basis for C4 AQO in SLE seems to be heterogeneous²¹ and linkage disequilibrium in Caucasians makes it difficult to assess the relative contribution of HLA and C4A*QO to disease susceptibility. Our data in this Tunisian population did not confirm the firmly established strong correlation between Ro(SSA)/La(SSB) autoantibodies and HLA DR/DQ haplotypes which have been found in SLE of Caucasian and African-American descent.

In conclusion, there is evidence from our results that genetic susceptibility to SLE among Tunisians appears similar to other ethnic lines. MHC class II alleles (DRB1, DQA1, DQB1) and C4 null associations that have been noted in other ethnic groups are also found in Tunisians, suggesting shared susceptibility factors across ethnic lines in predisposition to SLE.

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