Association of Selective HLA Class II Susceptibility-Conferring and Protective Haplotypes with Type 2 Diabetes in Patients from Bahrain and Lebanon

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The association of HLA class II with type 2 diabetes (T2DM) was investigated in Bahraini and Lebanese subjects. DRB1*070101 (Lebanese and Bahraini) and DQB1*0201 (Lebanese) were susceptibility-conferring alleles, and unique susceptibility-conferring/protective haplotypes were found in both patient groups. Regression analysis confirmed that DRB1*070101-DQB1*0201 (Bahraini) and DRB1*110101-DQB1*0201 (Lebanese) were susceptibility-conferring haplotypes.

Type 2 (non-insulin-dependent) diabetes mellitus (T2DM) is the most common diabetes form (19), and susceptibility to it is determined by environmental and genetic factors (9, 19), the latter being complex and poorly defined (5, 19). While the association of HLA class II genes in type 1 diabetes pathogenesis was reported for several ethnicities (1, 14), studies on HLA class II association with T2DM provided inconsistent results, since an association (16), no association (7), or a weak link between HLA class II and T2DM has been reported. A number of studies focused on the association of HLA with T2DM morbidity and mortality (17), highlighted by increased frequency of HLA-DR3 and -DR4 in islet cell autoantibody (ICA)-positive patients refractory to oral anti-diabetic drugs as reported by some (11) but not others (20), and association of HLA-DRB1*1502 with T2DM in anti-glutamic acid decarboxylase (GAD)-positive patients (10).

We previously reported on the distribution of HLA class II alleles and haplotypes among T2DM patients in the Bahraini population (16), an Arab Peninsula population with a high prevalence (24% of the adult population) of T2DM (2). In view of the heterogeneity of Arabs with distinct ethnic backgrounds and racial origins (3), this study addresses the association of HLA-DRB1 and HLA-DQB1 haplotypes with T2DM in Bahraini and Lebanese Arabs.

T2DM patients comprised 115 Lebanese (59 males and 56 females; mean age, 55.2 ± 13.5 years) and 110 Bahraini (59 males and 51 females; mean age, 52.3 ± 9.6 years) unrelated patients. Exclusion criteria included other types of diabetes, autoimmune diseases, and positive anti-GAD, anti-ryosine phosphatase-related protein (IA-2), or ICA autoantibody responses. Family history of diabetes (80/110 Bahraini patients and 77/115 Lebanese patients) or body mass index did not influence selection of subjects. The control group included 121 Lebanese (57 males and 64 females; mean age, 54.5 ± 13.8 years) and 154 Bahraini (79 males and 75 females; mean age, 52.9 ± 9.0 years) subjects with normal fasting/random glucose levels and no known personal or family history of diabetes. Demographic details, which included duration, first-degree family history, complications, and treatment for diabetes, were recorded. The Arabian Gulf University Ethics Committee approved the study (which was done according to Helsinki guidelines), and informed consent was obtained from all participants.

Total genomic DNA was extracted from EDTA-anticoagulated venous blood by the phenol-chloroform method. HLA-DRB1 and HLA-DQB1 gene alleles were analyzed using the PCR sequence-specific priming (SSP) technique, using an SSP2L HLA class II genotyping kit according to the manufacturer's specifications (One Lambda, Thousand Oaks, CA). Allele frequencies were determined by the gene counting method, and haplotype frequencies were determined by the maximum likelihood method, using the Arlequin (version 2.000) population analysis software. P values were corrected for the number of alleles tested (Pc) using Bonferroni's inequality method; significance was determined at a P value of <0.05.

Significant DRB1 and DQB1 allelic differences were seen between Lebanese and Bahraini T2DM patients and controls. When Bonferroni’s correction was applied, DRB1*070101 was significantly more common in both patient groups. While DQB1*0201 was significantly more common among Lebanese patients, none of the DQB1 loci were found to be significantly different between Bahraini patients and controls (Table 1). DRB1-DQB1 haplotype analysis showed that the frequency of DRB1*110101-DQB1*0201 was higher, while the frequencies of DRB1*040101-DQB1*050101, DRB1*110101-DQB1*030101, DRB1*130101-DQB1*060101, and DRB1*140101-DQB1*050101 were lower in Lebanese patients than in controls (Table 2). Logistic regression analysis demonstrated, after controlling...
haplotypes were seen among Bahraini and Lebanese T2DM patients, respectively (Table 3). Other haplotypes served a dominant role among Bahraini subjects confirmed our previous finding (16), and protective among their Lebanese counterparts. DRB1*110101-DQB1*0201 served a susceptibility-conferring role among Lebanese patients but was largely protective among Bahraini patients. The susceptibility conferred by DRB1*040101-DQB1*0302 and DRB1*070101-DQB1*0201 haplotypes among Bahraini subjects confirmed our previous finding (16),

### Table 1. HLA-DRB1* allele distribution among T2DM patients and controls

| Locus  | Bahraini subjects | Lebanese subjects |
|--------|-------------------|-------------------|
|        | Patients          | Controls          |        | Patients          | Controls          |
|        | Allele frequency |        |       | Allele frequency |       |
| DRB1   |                   | Pc              |       |                   | Pc              |
| 010101 | 0.014 ± 0.01      | 0.065 ± 0.01    | 3.591 | 0.044 ± 0.01      | 0.074 ± 0.02    | 3.734 |
| 030101 | 0.123 ± 0.02      | 0.149 ± 0.02    | 9.620 | 0.078 ± 0.02      | 0.046 ± 0.01    | 4.650 |
| 040101 | 0.109 ± 0.02      | 0.223 ± 0.01    | 3.482 | 0.183 ± 0.03      | 0.157 ± 0.02    | 6.337 |
| 070101 | 0.200 ± 0.03      | 0.084 ± 0.02    | 0.001 | 0.165 ± 0.02      | 0.070 ± 0.02    | 0.018 |
| 100101 | 0.055 ± 0.02      | 0.088 ± 0.02    | 2.014 | 0.026 ± 0.01      | 0.033 ± 0.01    | 16.121 |
| 110101 | 0.086 ± 0.02      | 0.136 ± 0.02    | 1.067 | 0.222 ± 0.03      | 0.322 ± 0.03    | 0.481 |
| 120101 | 0.018 ± 0.01      | 0.026 ± 0.01    | 15.742| 0.017 ± 0.01      | 0.029 ± 0.01    | 7.641 |
| 130101 | 0.018 ± 0.01      | 0.036 ± 0.01    | 4.950 | 0.074 ± 0.02      | 0.058 ± 0.02    | 6.685 |
| 140101 | 0.027 ± 0.01      | 0.023 ± 0.01    | 16.202| 0.017 ± 0.01      | 0.054 ± 0.01    | 0.588 |
| 150101 | 0.159 ± 0.03      | 0.088 ± 0.02    | 1.353 | 0.057 ± 0.02      | 0.062 ± 0.02    | 17.832 |
| 160101 | 0.077 ± 0.02      | 0.110 ± 0.02    | 3.435 | 0.039 ± 0.01      | 0.037 ± 0.01    | 17.302 |
| DQB1   |                   |                 |       |                   |                 |
| 0201   | 0.305 ± 0.03      | 0.273 ± 0.03    | 3.916 | 0.209 ± 0.03      | 0.107 ± 0.02    | 0.005 |
| 030101 | 0.132 ± 0.02      | 0.182 ± 0.02    | 1.032 | 0.261 ± 0.03      | 0.347 ± 0.03    | 0.198 |
| 0302   | 0.100 ± 0.02      | 0.055 ± 0.01    | 0.127 | 0.174 ± 0.03      | 0.153 ± 0.02    | 2.867 |
| 0401   | 0.041 ± 0.01      | 0.039 ± 0.01    | 6.125 | 0.035 ± 0.01      | 0.041 ± 0.01    | 4.936 |
| 050101 | 0.236 ± 0.03      | 0.276 ± 0.03    | 2.212 | 0.148 ± 0.02      | 0.215 ± 0.03    | 1.464 |
| 060101 | 0.159 ± 0.02      | 0.136 ± 0.02    | 5.696 | 0.113 ± 0.02      | 0.107 ± 0.02    | 5.034 |

### Table 2. DRB1*–DQB1* haplotype distribution

| Haplotype  | Distribution for each group | OR (95% CI)    | Distribution for each group | OR (95% CI)    |
|------------|-----------------------------|----------------|-----------------------------|----------------|
|            | Patients Controls           |                | Patients Controls           |                |
| DRB1*010101-DQB1*050101 | 0.0137 0.0328 | 0.41 (0.13–1.55) | 0.0284 0.0532 | 0.55 (0.23–1.42) |
| DRB1*030101-DQB1*0201  | 0.1136 0.1394 | 0.79 (0.47–1.34) | 0.0726 0.0422 | 1.85 (0.78–4.45) |
| DRB1*040101-DQB1*0302  | 0.0691 0.0250 | 3.37 (1.54–7.83) | 0.1316 0.1255 | 0.71 (0.42–1.19) |
| DRB1*070101-DQB1*0201  | 0.0665 0.0673 | 0.93 (0.47–1.87) | 0.0835 0.0490 | 1.73 (0.82–3.54) |
| DRB1*100101-DQB1*030302| 0.0182 0.0065 | 2.83 (0.54–12.08) | 0.0458 0.0158 | 2.99 (0.92–8.38) |
| DRB1*110101-DQB1*0201  | 0.0318 0.0780 | 0.39 (0.18–0.84) | 0.1314 0.0522 | 2.64 (1.33–5.04) |

### Notes

a HLA-DRB1* and HLA-DQB1* alleles were assessed by PCR-SSP, and haplotype frequencies were determined by the maximum likelihood method.
b Lebanese subjects comprised 115 T2DM patients and 121 controls; Bahraini subjects comprised 110 T2DM patients and 154 controls.
c Numbers in boldface indicate significant differences.
d OR, odds ratio; CI, confidence interval.
TABLE 3. Multinomial regression analysis

| Population and haplotype | P | ORa | 95% CI |
|--------------------------|---|-----|--------|
| **Bahraini**              |   |     |        |
| DRB1*070101-DQB1*0201    | <0.001 | 3.243 | 1.723–6.106 |
| DRB1*040101-DQB1*0302    | 0.057 | 2.186 | 0.976–4.897 |
| DRB1*110101-DQB1*0301    | 0.233 | 0.653 | 0.324–1.315 |
| **Lebanese**             |   |     |        |
| DRB1*040101-DQB1*050101  | 0.118 | 6.397 | 0.626–65.348 |
| DRB1*110101-DQB1*0201    | 0.045 | 2.894 | 1.025–8.172 |
| DRB1*110101-DQB1*030101  | 0.070 | 0.592 | 0.336–1.044 |
| DRB1*130101-DQB1*060101  | 0.172 | 2.092 | 0.725–6.032 |
| DRB1*140101-DQB1*050101  | 0.152 | 0.419 | 0.127–1.377 |

Note: Figures in bold indicate significant differences.

- a Potentially confounding variables controlled included, age, gender, body mass index, fasting glucose, glycosylated hemoglobin, and age at disease onset.
- b OR, odds ratio.
- c CI, confidence interval.
- d Numbers in boldface indicate significant differences.