Autoimmune Type 1 Diabetes Genetic Susceptibility Encoded by Human Leukocyte Antigen DRB1 and DQB1 Genes in Tunisia

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Received 9 March 2009/Returned for modification 12 June 2009/Accepted 15 June 2009

Type 1 (insulin-dependent) diabetes (T1D), and susceptible alleles and haplotypes were implicated in the pathogenesis of TID. This study investigated the heterogeneity in HLA class II haplotype distribution among Tunisian patients with TID. This was a retrospective case control study done in Monastir in central Tunisia. The subjects comprised 88 T1D patients and 112 healthy controls. HLA-DRB1 and -DQB1 genotyping was done by PCR-sequence-specific priming. Significant DRB1 and DQB1 allelic differences were seen between T1D patients and controls; these differences comprised DRB1*030101 and DRB1*0302, which were higher in T1D patients than in control subjects, and DRB1*070101, DRB1*110101, DQB1*030101, and DQB1*060101, which were lower in T1D patients than in control subjects. In addition, the frequencies of DRB1*030101-DQB1*0201 and DRB1*040101-DQB1*0302 were higher in T1D patients than in control subjects, and the frequencies of DRB1*070101-DQB1*0201 and DRB1*110101-DQB1*030101 haplotypes were lower in T1D patients than in control subjects. Multiple logistic regression analysis revealed the positive association of DRB1*030101-DQB1*0201 and DRB1*040101-DQB1*0302 and the negative association of only DRB1*070101-DQB1*0201 haplotypes with TID. Furthermore, a significantly increased prevalence of DRB1*030101-DQB1*0201 homozygotes was seen for TID subjects than for control subjects. Our results confirm the association of specific HLA-DR and -DQ alleles and haplotypes with TID in Tunisians. The identification of similar and unique haplotypes in Tunisians compared to other Caucasians highlights the need for evaluating the contribution of HLA class II to the genetic susceptibility to TID with regard to haplotype usage and also to ethnic origin and racial background.

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Published ahead of print on 24 June 2009.

MATERIALS AND METHODS

Subjects. Study subjects comprised 88 unrelated T1D patients (44 males and 44 females; age [mean ± standard error], 16.4 ± 7.7 years). The diagnosis of TID was according to both clinical features and laboratory data. All T1D patients...
were ketosis prone, lacked endogenous insulin secretion, and needed insulin for controlling hyperglycemia. TID patients were not obese, were free of any concomitant complication, and were not receiving additional treatment at the time of blood collection. Patients with other forms of diabetes were excluded. Control subjects consisted of 112 university students and healthy children (65 males and 47 females; age [mean ± standard error], 28.2 ± 5.8 years), who had normal glucose tolerance and no family history of TID or other autoimmune diseases. All patients and control subjects were Tunisian Arabs, were from central Tunisia, and were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

**HLA-DRB1 and -DQB1 genotyping.** HLA-DRB1 and -DQB1 alleles were analyzed using the PCR-sequence-specific priming technique, using the Micro SSP generic HLA class II (DRB/DQB) DNA typing kit (lot 05A), according to the manufacturer’s specifications (One Lambda, Thousand Oaks, CA). PCR products were analyzed on ethidium bromide-stained agarose gels. HLA allele nomenclature was as previously reported (23). In total, 16 DRB1 and 7 DQB1 products were analyzed on ethidium bromide-stained agarose gels. HLA allele frequencies were determined by the gene counting method (31), using the HLAStat 2000 software, which also computed the chance finding, even after Bonferroni’s correction. These comprised DRB1*030101 (Pc = 0.006), which was higher among TID patients, and DRB1*070101 (Pc = 0.003) and DRB1*110101 (Pc = 0.027), which were higher in control subjects (Table 1). Similarly, significant DQB1 allelic differences were seen at the DQB1 locus, three of seven loci being significantly different even after Bonferroni’s correction. These comprised DQB1*030101 (Pc = 0.012), which was higher among TID patients, and DQB1*030101 (Pc = 0.007) and DQB1*060101 (Pc = 0.041), which were higher among control subjects (Table 2).

### RESULTS

**HLA-DRB1 and HLA-DQB1 allele frequencies.** Significant DRB1 allelic differences were seen between TID patients and controls, with 6 of 16 loci being significantly different (P < 0.05). When Bonferroni’s correction was applied, differences were significant for only three loci, which comprised DRB1*030101 (Pc = 0.006), which was higher among TID patients, and DRB1*070101 (Pc = 0.003) and DRB1*110101 (Pc = 0.027), which were higher in control subjects (Table 1).

### TABLE 1. HLA-DRB1* allele distribution

| DRB1* allele | TID patients (n = 88) Mean allele frequency SE | Controls (n = 112) Mean allele frequency SE | Pc | Pc c | OR |
|--------------|-----------------------------------------------|-------------------------------------------|-----|------|----|
| 010101       | 0.0455 0.0157                                 | 0.0804 0.0182                               |     |      |    |
| 030101       | 0.3636 0.0363                                 | 0.1473 0.0237                               |     |      |    |
| 020101       | 0.0000 0.0000                                 | 0.0013 0.0077                               |     |      |    |
| 0317         | 0.0227 0.0112                                 | 0.0019 0.0088                               |     |      |    |
| 040101       | 0.2386 0.0321                                 | 0.1161 0.0214                               |     |      |    |
| 070101       | 0.0682 0.0190                                 | 0.2143 0.0274                               |     |      |    |
| 080101       | 0.0170 0.0098                                 | 0.0223 0.0099                               |     |      |    |
| 090102       | 0.0284 0.0125                                 | 0.0000 0.0000                               |     |      |    |
| 100101       | 0.0227 0.0112                                 | 0.0025 0.0162                               |     |      |    |
| 110101       | 0.0284 0.0125                                 | 0.1071 0.0207                               |     |      |    |
| 120101       | 0.0170 0.0098                                 | 0.0045 0.0045                               | 0.207 | 3.313 | 3.918 |
| 130101       | 0.0966 0.0223                                 | 0.1250 0.0221                               | 0.248 | 3.967 | 0.667 |
| 1302         | 0.0000 0.0000                                 | 0.0045 0.0045                               | 1.000 | 16.000 | 0.000 |
| 140101       | 0.0000 0.0000                                 | 0.0134 0.0077                               | 0.122 | 1.950 | 0.000 |
| 150101       | 0.0227 0.0112                                 | 0.0625 0.0162                               | 0.075 | 1.208 | 0.363 |
| 160101       | 0.0284 0.0125                                 | 0.0089 0.0063                               | 0.137 | 2.187 | 3.313 |

*a* P values were determined by Fisher’s exact test. Boldface values indicate that there were significant differences between the mean allele frequencies for the patients with TID and controls.

*b* Pc, corrected P values for the number of alleles tested, calculated using the Bonferroni method.

### TABLE 2. HLA-DQB1* allele distribution

| DQB1* allele | Patients (n = 88) Mean allele frequency SE | Controls (n = 112) Mean allele frequency SE | Pc | Pc c | OR |
|--------------|-----------------------------------------------|-------------------------------------------|-----|------|----|
| 0201         | 0.4261 0.0373                                 | 0.2902 0.0303                               |     |      |    |
| 030101       | 0.0966 0.0223                                 | 0.2009 0.0268                               |     |      |    |
| 0302         | 0.2159 0.0310                                 | 0.0938 0.0195                               |     |      |    |
| 030302       | 0.0739 0.0197                                 | 0.0536 0.0150                               |     |      |    |
| 0401         | 0.0227 0.0112                                 | 0.0625 0.0162                               |     |      |    |
| 050101       | 0.0852 0.0210                                 | 0.1205 0.0218                               |     |      |    |
| 060101       | 0.0795 0.0204                                 | 0.1786 0.0256                               |     |      |    |

*a* P values were determined by Fisher’s exact test.

*b* Pc, corrected P values for the number of alleles tested, calculated using the Bonferroni method.
Frequencies of DRBI-DQB1 haplotypes. Of the eight frequent haplotype identified, the frequencies of DRBI*030101-DQB1*0201 (Pc < 0.001), and DRBI*040101-DQB1*0302 (Pc = 0.010) were higher among T1D patients, thereby conferring T1D susceptibility to these haplotypes (Table 3). In addition, the frequencies of DRBI*070101-DQB1*0201 (Pc = 0.015) and DRBI*110101-DQB1*0301 (Pc = 0.036) were lower in T1D patients than in control subjects, thus assigning a disease-protective nature to these haplotypes (Table 3).

Regression analysis. The contribution of specific HLA DRBI-DQB1 to T1D was analyzed by multiple logistic regression analysis. Logistic regression analysis confirmed that DRBI*030101-DQB1*0201 (OR, 3.88; 95% confidence interval [95% CI], 1.88 to 8.02) and DRBI*040101-DQB1*0302 (OR, 2.91; 95% CI, 1.35 to 6.23) were positively associated, while DRBI*070101-DQB1*0201 (OR, 0.37; 95% CI, 0.16 to 0.85) was negatively associated with T1D (Table 4). The initial negative association of DRBI*110101-DQB1*030101 with T1D was rejected according to the model employed.

Discussion

Results obtained demonstrated that the contribution of HLA haplotypes to T1D genetic susceptibility among Tunisians depends on specific HLA class II haplotypes. The DRBI*030101-DQB1*0201 haplotype fitted a recessive model, since it confers strong T1D susceptibility when present in a homozygous state (PC = 2.5 × 10^{-4}; OR = 43.79), rather than in a heterozygous state (PC = 0.089; OR = 5.19). The high T1D susceptibility conferred by both DRBI*030101-DQB1*0201 and DRBI*040101-DQB1*0302 haplotypes was reminiscent of previous studies of Caucasians (3, 13, 16, 19), but not non-Caucasians (12), supporting the notion of Caucasian T1D susceptibility haplotypes.

DQB1*0302 was strongly associated with, while DQB1*0301 was largely protective of T1D. Similar associations were reported for northern Europe (8, 14–16), but not southern Europe (15, 24) or Mediterranean countries (3), in which DQB1*0201 was reported as the major DQB1 susceptible allele. This lack of association of DQB1*0201 with T1D in Tunisians was supported by the finding that DQB1*0201 was linked with T1D susceptibility when present with DRBI*030101 but was negatively associated with T1D when present with DRBI*070101 in a haplotype. Thus, it appears that DQB1*0201 did not play a significant role in T1D pathogenesis and that the disease protection or susceptibility may be explained the presence of DQB1*0201 haplotypes with protective or susceptible DRBI alleles, respectively, as was also suggested elsewhere (10).

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DQB1*030101-DQB1*0201 and DRBI*040101-DQB1*0302 were strongly associated with, while DRBI*070101-DQB1*0201 was protective of T1D, further supporting the notion that DRBI*030101-DQB1*0201 on its own is a major T1D susceptibility haplotype among Caucasians (3, 13, 19). Our findings were reminiscent of earlier studies of Tunisians, which showed that DR3 and DR4 (1, 5) and DRBI*03-DQB1*0201 and DRBI*04-DQB1*0302 haplotypes (1) were strongly associated with T1D. The notable difference was the identification of DRBI*070101-DQB1*0201 as the T1D-protective haplotype in our study, compared to DRBI*1501-DQB1*0602 reported by Abid Kamoun (1). While explanation for these apparent
discrepancies remain speculative at this stage, it is likely due to sample size differences, selection of study subjects, and the failure to control for potential covariates by earlier studies (1, 5).

The identification of DRB1*030101-DQB1*0201 and DRB1*040101-DQB1*0302 as T1D-susceptible haplotypes and of DRB1*070101-DQB1*0201 and DRB1*110101-DQB1*030101 as T1D-protective haplotypes was comparable to previous results with Caucasians (3, 13, 19, 21). Of these haplotypes, regression analysis rejected DRB1*110101-DQB1*030101 as a T1D-protective haplotype, and its presence in a homozygous or heterozygous state did not impart any T1D protection aspect according to the model employed. Previous identification of low-risk or protective haplotypes, including DRB1*110101-DQB1*030101, may imply protection, or may be the consequence of reduction in its frequency in T1D patients brought about by corresponding increase in frequency of susceptibility haplotypes (DRB1*040101-DQB1*0302 and DRB1*030101-DQB1*0201), as was suggested elsewhere (10). Collectively, this supports the notion of intricate interplay between individual DRB1 and DQB1 loci in determining susceptibility to T1D.

HLA class II DR and DQ complex bind antigen fragments and directly present the antigen to T cells. The presence (or absence) of certain residues within the peptide-binding sites of the DR-DQ complex was suggested to dictate the predisposition to or protection from disease, including T1D (10, 20). By binding β-cell-specific peptides in the context of peptide-major histocompatibility complex complex, specific class II haplotypes are involved in activation and later expansion of autoreactive T cells (2, 9, 11). As such, the strong association of DRB1*030101-DQB1*0201 and DRB1*040101-DQB1*0302 and the negative association of DRB1*070101-DQB1*0201 with T1D may be explained by differences in affinity to (autoantigenic) peptide fragments presented by each haplotype. This may involve fitting of these peptide fragments within the respective haplotype binding grooves and would be useful in the screening of additional autoantigens linked with diabetes and in the identification of specific epitopes likely to interact with diabetogenic autoreactive T cells (2, 6).

The identification of positive and negative association of specific HLA class II haplotype to T1D pathogenesis may have important clinical implications, likely by allowing identification of at-risk individuals, and thus early intervention. However, despite the strength of the association observed, our study has some limitations, namely, that it was limited to the HLA DRB and DQB regions, and thus did not allow for examination of the possible association of additional HLA loci, or other genes in linkage disequilibrium with HLA alleles, with T1D. Our results highlight the significance of analyzing haplotypes and genotypic combinations, as opposed to single alleles, in assigning T1D genetic susceptibility. Accordingly, a specific haplotype may modulate the susceptible/protective nature of another haplotype within a particular genotypic combination.

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TABLE 4a. Genotypic combination of DRB1-DQB1 haplotypes

| DRB1*030101-DQB1*0201 | % Patients | % Controls | PS  | Pc  | OR  |
|-----------------------|------------|------------|-----|-----|-----|
| DRB1*040101-DQB1*0302 | 6 (6.8)    | 0 (0.0)    | 0.017 | 0.097 | 17.73 |
| DRB1*030101-DQB1*0201 | 11 (12.5)  | 3 (2.7)    | 0.015 | 0.087 | 5.19 |
| DRB1*110101-DQB1*030101 | 2 (2.3)  | 3 (2.7)    | 0.784 | 1.000 | 1.000 |
| DRB1*070101-DQB1*0201 | 1 (1.1)    | 6 (5.4)    | 0.221 | 0.777 |       |

PS = 0.221; Pc = 0.777; OR = 5.19. The percentage of total within group is shown in parentheses.

p values were determined by Fisher’s exact test.

P values adjusted using the Sidak correction factor.

DISCLOSURE STATEMENT

None.

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