HLA-DQB1* alleles and genetic susceptibility to type 1 diabetes mellitus

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Abstract

AIM: To determine human leukocyte antigen (HLA)-DQB1 allele association with susceptibility to type 1 diabetes (T1D) and to clinical and laboratory findings.

METHODS: This study was conducted on 85 unrelated Egyptian children with T1D recruited consecutively from the Pediatric Diabetes Endocrinology outpatients Clinic; Mansoura University Children’s Hospital, Egypt. Patient mean follow up period was 2.5 years. Patients were subdivided according to level of HbA1c (optimal/suboptimal control < 8.5% and poor control ≥ 8.5%). The control group consisted of 113 unrelated age- and sex-matched healthy subjects without T1D or other autoimmune diseases. Genomic DNA extraction was done for all subjects using a DNA isolation kit. HLA-Class II-DQB1 allele typing was carried out with a polymerase chain reaction-sequence-specific oligonucleotide probe using an INNO-LIPA HLA-DQB1 update kit.

RESULTS: Significant differences were detected between Egyptian patients with T1D and control groups in the frequencies of DQB1*02 [44.4% vs 18.6%, corrected P value (Pc) < 0.001] and DQB1*03 (41.2% vs 24.4%, Pc < 0.001). Significant differences were also observed between control groups and T1D patients in the frequencies of DQB1*05 (14.6% vs 7.2%, P = 0.029) and DQB1*06 (34.1% vs 7.2%, P < 0.001). However, after correction for multiple comparisons, the significance was retained for HLA-DQB1*06 (Pc < 0.001) but lost for HLA-DQB1*05. HLA-DQB1*0201, *0202, *030201 were positively associated with T1D (Pc = 0.014, Pc < 0.001, and Pc < 0.001 respectively), while HLA-DQB1*060101 was negatively associated (Pc < 0.001) with the condition. Although the HLA-DQB1 alleles 030101 and 050101 were significantly higher in controls (P = 0.016, P = 0.025 respectively), both of them lost statistical significance after correction of P value. The frequency of the HLA-DQB1 genotypes 02/02, 02/03, and 03/03 was higher in T1D patients, and the frequency of the genotypes 03/06, 05/06, and 06/06 was higher in controls, these differences being statistically significant before correction. After correction, the genotypes 02/02, 02/03 in T1D, and the genotypes 03/06, 05/06, and 06/06 in controls were still significant (Pc = 0.01, Pc < 0.001, and Pc < 0.001 respectively). Non-significant associations were found between the frequency HLA-DQB1 alleles and genotypes in T1D in relation to the grade of diabetic control, Microalbuminuria, age, gender, age of presentation, weight, height, frequency of diabetic ketoacidosis (P =
Type 1 diabetes (T1D) mellitus is an organ-specific autoimmune disease characterized by T-cell-mediated destruction of pancreatic islets\cite{10,12}. This study was conducted on 85 unrelated Egyptian children with T1D recruited consecutively from the Pediatric Diabetes Endocrinology outpatient Clinic; Mansoura University Children’s Hospital, Egypt. Studied patients were 35 males and 50 females. Patient mean age range was 12.52 ± 2.98 years (range 3.5-16 years) with mean age of presentation 8.5 ± 3.1 years. Patient mean follow up period was 2.5 years (range 1-6 years). Patients were subdivided according to level of HbA1c (optimal/suboptimal control < 8.5% and poor control ≥ 8.5%)\cite{25,26}.

All patients were treated by basal-bolus insulin regimen (3 rapid acting human insulin does as a bolus dose before the main meals and one intermediate acting human insulin does at bed time). Serum cholesterol measurement was carried out for all patients after overnight fast for 8-12 h. Patients were diagnosed with microalbuminuria if two of three consecutive urine samples showed elevated albumin excretion\cite{27}.

The control group consisted of 113 unrelated age- and sex-matched healthy subjects without TID or other autoimmune diseases such as autoimmune thyroid disease, living in the same geographical area and with the same ethnic origin as patients. Written informed consent was obtained from the parents of patients and controls after approval of the study protocol by the local ethical committee.

### HLA class II - DQB1 allele typing

Genomic DNA extraction was done for all samples using a DNA isolation kit (QIAmp DNA blood mini kit Cat. 51104, Qiagen, Gmbh). HLA-Class II -DQB1 allele typing was done carried out with a polymerase chain reaction-sequence-specific oligonucleotide probe using an INNO-LiPA HLA-DQB1 update kit (Lot number 152003, Innogenetics, Belgium). Test conditions were according to manufacturer’s instruction.
Statistical analysis
To compare the frequency of HLA-DQB1 alleles in children with T1D and controls, the conventional \( \chi^2 \) test with Yates’ correction for continuity, when appropriate, was used. SPSS version 17 was used for statistical analysis. The odds ratio (OR) was calculated with \( 2 \times 2 \) contingency tables. The 95% confidence intervals were obtained using Cornfield’s approximation. Data was analyzed by one-way ANOVA for multiple comparisons. The \( P \) value was corrected (\( P_c \)) for the number of alleles tested. The level of significance was set at 95%. \( P \) value less than 0.05 was considered significant.

RESULTS
The average weight and height of studied patients were 44.55 ± 14.51 kg and 144.6 ± 15.55 cm respectively. Microalbuminuria was found in only 5 patients. The average serum cholesterol level was 163.95 ± 28.1, and the HbA1c average level was 7.98 ± 1.59. Optimal/suboptimal control of HbA1c level was found in 57 patients, and poor control in 28 patients (Table 1).

Significant differences were detected between Egyptian patients with T1D and control groups in the frequencies of DQB1*02 (44.4% vs 18.6%, \( P_c < 0.001 \), OR: 3.5) and DQB1*03 (41.2% vs 24.4%, \( P_c < 0.001 \), OR: 2.17). Significant differences were also observed between control groups and T1D patients in the frequencies of DQB1*05 (14.6% vs 7.2%, \( P = 0.029 \), OR: 0.45) and DQB1*06 (34.1% vs 7.2%, \( P < 0.001 \), OR: 0.15). However, after correction for multiple comparisons, the significance was retained for HLA-DQB1*06 (\( P_c < 0.001 \)) but lost for HLA-DQB1*05.

From analysis of the frequency of allele subtypes in T1D patients and controls, HLA-DQB1*0201, *0202, *030201 were found to be positively associated with T1D (\( P_c = 0.014 \), \( P_c < 0.001 \), and \( P_c < 0.001 \) respectively), while HLA-DQB1*060101 was negatively associated (\( P_c < 0.001 \)). Although the HLA-DQB1 alleles 030101 and 050101 were significantly higher in controls (\( P = 0.016 \), \( P = 0.025 \) respectively), both of them lost statistical significance after correction of \( P \) value (Table 2).

From analysis of HLA-DQB1 genotypes in T1D patients and controls, the frequency of the genotypes 02/02, 02/03, and 03/03 was found to be higher in T1D patients, and the genotypes 03/06, 05/06, and 06/06 was higher in controls, with these differences being statistically significant before correction. After correction, the genotypes 02/02, 02/03 in T1D, and the genotypes 03/06, 06/06 in controls still showed significant differences (\( P_c = 0.01 \), \( P_c < 0.001 \), \( P_c < 0.001 \), and \( P_c = 0.04 \), respectively) (Table 3).

In the analysis of the frequency HLA-DQB1 alleles and genotypes in T1D in relation to grade of diabetic control (Table 4), Microalbuminuria, age, gender, age of presentation, weight, height, frequency of diabetic ketoacidosis (\( P = 0.42 \)), serum cholesterol, and level of fasting and post-prandial C-peptide (\( P = 0.83 \), \( P = 0.9 \), respectively), only non-significant associations were found (data not shown).

DISCUSSION
T1D mellitus is a chronic disease which most frequently presents in childhood[28,29]. It is classified into type 1B (idiopathic) and 1A diabetes mellitus, mediated through the immune system[30,31]. In T1D 1A, a genetically susceptible individual presents with loss of tolerance to the pancreatic islet tissue triggered by environmental factors[32] and develops a progressive, immune-mediated destruction of pancreatic islet \( \beta \) cell[31,32].

T1D is considered a multifactorial condition with complex interactions between genetic and environmental factors[28,31]. There is evidence showing that 40%-50% of the inherited susceptibility to the disease is contributed by HLA-DR-DQ[33]. The association of specific HLA-DQB1 alleles and genotypes with T1D susceptibility/protection depends on the ethnicity and racial background of each population. For example, in Caucasians T1D is positively associated with DQB1*0201 and DQB1*0302, while in Japanese it is associated with DQB1*0401 and DQB1*0303.

From the results of the current study, significant positive associations were found with HLA-DQB1*02 and DQB1*03 (\( P_c < 0.001 \), OR = 3.5, OR = 2.17 respectively) and a negative association with DQB1*06 (\( P < 0.001 \), OR: 0.15) in Egyptian children with T1D. At the same time, HLA-DQB1*0201, *0202, *030201 were positively associated (\( P_c = 0.014 \), \( P_c < 0.001 \), \( P_c < 0.001 \) respectively), and HLA-DQB1*060101 was negatively associated (\( P_c < 0.001 \)) with T1D. The strongest positive association was found for HLA-DQB1*030201, followed by *0202, and finally *0201 (OR = 19.2, OR = 14.4, and OR = 2.21, respectively). To the best of our knowledge, the present study is the first to identify a positive association between

**Table 1  Clinical and laboratory characteristics of type 1 diabetes mellitus patients**

| Characteristic                      | % (%)         |
|------------------------------------|---------------|
| Age (mean ± SD, yr)                | 12.52 ± 2.99  |
| Age of presentation (mean ± SD, yr)| 8.5 ± 3.1     |
| Gender: mean/female                | 35 (41.2)/50 (58.8) |
| Weight (kg)                        | 44.55 ± 14.51 |
| Height (cm)                        | 144.6 ± 15.35 |
| Microalbuminuria                   |               |
| Yes                                | 80 (94.1)     |
| No                                 | 5 (5.9)       |
| Frequency of DKA                   |               |
| Yes                                | 1.22 ± 0.91   |
| No                                 | 0.34 ± 0.28   |
| C-peptide (fasting)                |               |
| Yes                                | 0.58 ± 0.64   |
| No                                 | 0.34 ± 0.28   |
| C-peptide (post-prandial)          |               |
| Yes                                | 163.95 ± 28.1 |
| No                                 | 7.98 ± 1.59   |
| Serum cholesterol                  |               |
| Yes                                | 57 (67.1)     |
| No                                 | 80 (94.1)     |
| Grades of HbA1c control            |               |
| Optimal/suboptimal                 | 57 (67.1)     |
| Poor                               | 28 (32.9)     |

The results of the current study, significant positive associations were found with HLA-DQB1*02 and DQB1*03 (\( P_c < 0.001 \), OR = 3.5, OR = 2.17 respectively) and a negative association with DQB1*06 (\( P < 0.001 \), OR: 0.15) in Egyptian children with T1D. At the same time, HLA-DQB1*0201, *0202, *030201 were positively associated (\( P_c = 0.014 \), \( P_c < 0.001 \), \( P_c < 0.001 \) respectively), and HLA-DQB1*060101 was negatively associated (\( P_c < 0.001 \)) with T1D. The strongest positive association was found for HLA-DQB1*030201, followed by *0202, and finally *0201 (OR = 19.2, OR = 14.4, and OR = 2.21, respectively). To the best of our knowledge, the present study is the first to identify a positive association between

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Table 2 HLA-DQB1 allele frequency in type-1 diabetes mellitus group vs control group n (%)  

| HLA-DQB1 allele | Patient (n = 85) | Control (n = 113) | OR     | 95% CI  | P value | Pc value |
|-----------------|-----------------|------------------|--------|---------|---------|----------|
| 02              | 68 (44.4)       | 38 (18.6)        | 3.5    | 2.19-5.65 | < 0.001 | < 0.001 |
| 0201            | 49 (32.0)       | 36 (16.7)        | 2.21   | 1.35-3.62 | 0.001   | NS       |
| 0202            | 19 (12.4)       | 2 (1.0)          | 14.4   | 3.3-62.8 | < 0.001 | < 0.001 |
| 03              | 63 (41.2)       | 50 (24.4)        | 2.17   | 1.38-3.41 | < 0.001 | < 0.001 |
| 030101          | 4 (2.6)         | 18 (8.8)         | 0.279  | 0.09-0.84 | 0.016   | NS       |
| 030201          | 56 (36.6)       | 6 (2.9)          | 19.2   | 7.9-45.9 | < 0.001 | < 0.001 |
| 04              | 0               | 17 (8.3)         | -      | -        | NS      | NS       |
| 05              | 11 (7.2)        | 30 (14.6)        | 0.45   | 0.22-0.93 | 0.029   | NS       |
| 050101          | 6 (3.9)         | 21 (10.2)        | 0.35   | 0.14-0.91 | 0.025   | NS       |
| 050201          | 6 (3.3)         | 1 (0.5)          | 6.9    | 0.79-59.6 | 0.043   | NS       |
| 06              | 11 (7.2)        | 70 (34.1)        | 0.15   | 0.07-0.29 | < 0.001 | < 0.001 |
| 060101          | 2 (1.3)         | 51 (24.9)        | 0.04   | 0.01-0.17 | < 0.001 | < 0.001 |
| 0603            | 4 (2.6)         | -                | 2.37   | 2.1-2.7  | 0.020   | NS       |
| 060401          | 4 (2.6)         | 1 (0.5)          | 5.48   | 0.64-49.5 | 0.090   | NS       |

HLA: Human leukocyte antigen; OR: Odds ratio; NS: Not significant; Pc value: P value corrected for 14 comparisons. Significant P value if < 0.05.

Table 3 HLA-DQB1 genotype¹ frequency in type 1 diabetes mellitus group vs control n (%)  

| HLA-DQB1 genotype | Patient (n = 85) | Control (n = 113) | OR     | 95% CI  | P value | Pc value |
|-------------------|-----------------|------------------|--------|---------|---------|----------|
| 02/02             | 14 (16.5)       | 3 (2.7)          | 7.23   | 2.01-26.1 | 0.001   | 0.01     |
| 02/05             | 38 (44.7)       | 8 (7.1)          | 10.61  | 4.56-24.5 | < 0.001 | < 0.001 |
| 02/04             | -               | 7 (6.3)          | 1.01   | 1.02-1.18 | 0.01    | NS       |
| 02/06             | 6 (7.1)         | 4 (3.5)          | 2.07   | 0.56-7.57 | 0.26    | NS       |
| 02/05             | 6 (7.3)         | 16 (14.2)        | 0.46   | 0.17-1.23 | 0.16    | NS       |
| 03/03             | 12 (14.1)       | 6 (5.3)          | 2.93   | 1.05-18.6 | 0.033   | NS       |
| 03/05             | 4 (4.7)         | 8 (7.1)          | 0.65   | 0.19-2.23 | 0.48    | NS       |
| 03/06             | 2 (2.4)         | 22 (19.5)        | 0.1    | 0.02-0.44 | < 0.001 | < 0.001 |
| 05/06             | 1 (1.2)         | 11 (9.7)         | 0.11   | 0.01-0.87 | 0.012   | NS       |
| 06/06             | 2 (2.4)         | 16 (14.2)        | 0.15   | 0.03-0.65 | 0.004   | 0.04     |

¹Genotypes with frequency more than 5%. HLA: Human leukocyte antigen; OR: Odds ratio; NS: Not significant; Pc value: P value corrected for 10 comparisons. Significant P value if < 0.05.

Table 4 HLA-DQB1 genotype¹ frequency in relation to grades of diabetic control n (%)  

| HLA-DQB1 genotype | Optimal control (n = 57) | Poor control (n = 28) | OR (95% CI) | P value |
|-------------------|--------------------------|-----------------------|-------------|---------|
| 02/02             | 10 (65.9)                | 4 (60.5)              | 1.277 (0.36-4.49) | 0.703   |
| 02/03             | 26 (34.1)                | 12 (39.5)             | 1.19 (0.45-2.79) | 0.810   |
| 02/05             | 4 (39.4)                 | 2 (28.9)              | 0.981 (0.18-5.71) | 0.983   |
| 02/06             | 5 (53.0)                 | 1 (63.2)              | 2.59 (0.29-23.35) | 0.379   |
| 03/03             | 7 (7.6)                  | 5 (7.9)               | 0.644 (0.19-2.25) | 0.488   |
| 03/05             | 3                        | 1                     | 1.5 (0.15-15.11) | 0.729   |
| 06/06             | 1                        | 1                     | 0.482 (0.03-8.01) | 0.603   |

¹Genotypes with frequency more than 5%. HLA: Human leukocyte antigen; OR: Odds ratio. Significant P value if ≤ 0.05. Optimal/suboptimal control < 8.5, poor control ≥ 8.5.

HLA-DQB1*0202 and T1D (Table 2).

DQB1*0202 and DQB1*0302 were positively associated with T1D in various ethnic populations including Asians³⁴,⁳⁵, European³⁶-⁴³, and Americans³⁴,⁴⁶. Similar results were reported for Arab patients from Saudi Arabia³⁴,⁴⁶, Kuwait, Tunisia³⁴, Lebanon³⁴, and Israel³⁵. On the other hand, DQB1*0301 and DQB1*0601 were negatively associated with T1D in Korean³⁴, Latin American³⁵, Lebanese³⁴, Tunisian³⁴, Saudi children³⁴, Turkey³⁴, and Romanian³⁴ populations.

In the literature, there is only one previous study investigating the association of HLA-DQB1 alleles with T1D in Egyptians. Gaber et al³⁵ reported that HLA-DQB1*0201/*0302 were risk factors and *0601/*0603 were protective alleles. The two studies agree in relation to *0201, *0302, and *0601, but differ regarding *0603 in Gaber et al³⁵, and *0202 in the present work. HLA-DQB1*0603 was not detected in the controls of the present work. However, the present work was done on a different number of samples (85 patients vs 50 patients and 113 controls vs 50 controls) and a different geographical area (Delta region vs Cairo) from Gaber et al³⁵.
Egyptian are known to be of mixed ethnic origin (Middle Eastern, African and European)\cite{55}, so Egyptian studies are expected to add to the data available for different ethnic background\cite{56}. To the best of our knowledge, the present study is the first one to mention the positive association between HLA-DQB1*0202 and T1D (Table 2).

These same HLA-DQ molecules are associated with diabetes risk in various Caucasian and black populations although their relative frequency in background populations varies. This is also reflected in genotypes found among T1D patients and comparison of high risk genotype frequencies is most relevant to disease susceptibility\cite{57}. In Egyptian children with T1D, the genotypes 02/02, 02/03 were positively associated and the genotypes 03/06, 06/06 were negatively associated with the disease ($P_e = 0.01$, $P_c < 0.001$, $P_c < 0.001$, and $P_c = 0.04$, respectively) with the highest risk being with the heterozygote DQB1*02/*03 genotype (OR = 10.6) (Table 3). Similar findings were reported in the United States\cite{58}, Hungary\cite{59}, Romania\cite{60}, and Saudi Arabia\cite{61,62}.

The inheritance of HLA genes associated with T1DM would involve the presentation of diabetic autoantigen to autoreactive T-cells, thereby launching a T-cell activation cascade and the subsequent destruction of pancreatic β islet cells\cite{63}. It is tempting, therefore, to speculate that the DQB1*0201, DQB1*0302 genotypes and the homozygote DQB1*0201 genotype predispose to the stimulation of auto-reactive T-cells, thereby precipitating β-cell-directed immunity. Individuals carrying DQB1*0301, DQB1*0602 or DQB1*0602 may have a reduced affinity for diabetic autoantigen peptides, thereby explaining the dominant, protective nature of these peptides\cite{64}.

Geoad-epidemiological studies have highlighted that there is considerable geographic and ethnic variability not only in the incidence of T1D and its genetic determinants, but also in the acute and long-term complications and the resulting mortality risk associated with the disease. Comparisons of the genetic determinants of T1D in various populations have provided some evidence that the worldwide variation in incidence is at least partially determined by differences in genetic risk factors\cite{65}. Our results showed no correlation between HLA-DQB1 and diabetic nephropathy as the number of patients with microalbuminuria was considered as a limiting factor. Ronningen et al\cite{66} also found no association between HLA class II alleles and microalbuminuria. No significant association was found between HLA-DQB1 and the degree of diabetic control. Further investigation of this issue in a large group of diabetic patients of matched age, sex, diet and lifestyle is needed.

The Current work suggests that HLA-DQB1 alleles *030201, *0202, *0201, and genotypes 02/03, 02/02 may be a susceptibility risk factors for development of T1D in Egyptian children, and HLA-DQB1*060101 allele, 03/06, 06/06 genotypes may be protective factors. HLA-DQB1 alleles and genotypes do not contribute to the grade of diabetic control.

**COMMENTS**

**Background**

The existence of regional differences in the prevalence and nature of diabetes-related human leukocyte antigen (HLA) haplo- and genotypes as a function of the incidence of the disease within Europe and other regions, necessitates the collection of HLA genotype data from the perspective of prediction and prevention studies at the regional or national level.

**Research frontiers**

A significant positive associations were found with HLA-DQB1*0202 and DQB1*03 and a negative association with DQB1*06. HLA-DQB1*0201, *0202, *030201 were positively associated, and HLA-DQB1*060101 was negatively associated with type 1 diabetes (T1D).

**Innovations and breakthroughs**

The strongest positive association was found for HLA-DQB1*030201, followed by *0202, and finally *0201. The present study may be the first to mention the positive association between HLA-DQB1*0202 and T1D.

**Applications**

No significant association was found between HLA-DQB1 and the degree of diabetic control. Further investigation of this issue in a large groups of diabetic patients of matched age, sex, diet and lifestyle is needed.

**Peer review**

The authors examined HLA-DQB1 allele association with susceptibility to T1D in a cohort of Egyptian children. They concluded that HLA-DQB1 alleles *030201, *0202, *0201, and genotypes 02/03, 02/02 may be susceptibility risk factors for development of T1D, and HLA-DQB1*060101 allele, 03/06, 06/06 genotypes may be protective factors. HLA-DQB1 alleles and genotypes do not contribute to the grade of diabetic control.

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