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ORIGINAL ARTICLE

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 a 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, 06/06 in controls were still significant (*Pc* = 0.01, *Pc* < 0.001, *Pc* < 0.001, and *Pc* = 0.04, 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* =

0.42), serum cholesterol, and fasting and post-prandial level of C-peptide (P = 0.83, P = 0.9, respectively).

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

Key words: HLA-DQB1; Type 1 diabetes; Egyptian; Genetic susceptibility; Children, Complication

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INTRODUCTION

Type 1 diabetes (T1D) mellitus is an organ-specific autoimmune disease characterized by T-cell-mediated destruction of pancreatic islets[1,2]. Both genetic and environmental factors are involved in the pathogenesis of the autoimmune process leading to the onset of this disease[3-6].

Both genome screens and studies searching candidate genes have confirmed that T1D is a heterogeneous polygenic disorder, with about 20 loci contributing to the susceptibility to disease[7-9]. It is believed that the most important genes, responsible for more than of 50% genetic risk of developing diabetes, are located in human leukocyte antigen (HLA) region on chromosome 6[10].

Although HLA class I may significantly influence the overall risk for diabetes[10], the HLA class Ⅱ loci DQA1, DQB1, and DRB1 contribute most to the genetic predisposition to T1D[11-14]. Their analysis remains the cornerstone of genetic risk stratification in the framework of diabetes prevention studies in risk groups such as family members of patients[15] or in the general population[16].

Furthermore, the existence of regional differences in the prevalence and nature of diabetes-related HLA haplo- and genotypes as a function of the incidence of the disease within Europe and other regions[17-20], necessitates the collection of HLA genotype data in the perspective of prediction and prevention studies at the regional or national level[21].

The most relevant non-HLA genes identified as susceptible for T1D are those connected with the T-cell-mediated immune response. The activity level of T-cells and their effector functions are determined by intracellular signaling pathways and related genes. These include PTPN22 and CTLA-4, both of which prevent spontaneous activation of auto-reactive cells and development of autoimmunity[22,23]. In Egyptian children, the CTLA-4 +49 GG homozygous genotype is especially associated with T1D in younger patients and with younger age of onset, while the AG heterozygote genotype is associated with moderate or poor control of T1D[24].

This study set out to determine HLA-DQB1 allele association with susceptibility and/or protection to T1D and with clinical and laboratory findings in a cohort of Egyptian children.

MATERIALS AND METHODS

Patients and healthy controls

T1D mellitus is an organ-specific autoimmune disease characterized by T-cell-mediated destruction of pancreatic islets[1,2]. 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. 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%)[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[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, Qaigene, Gmbh). HLA-Class Ⅱ-DQB1 allele typing was done carried out with a polymerase chain reaction-sequence-specific oligonucleotide probe using a 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 χ^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 × 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 (*Pc*) 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%, *Pc* < 0.001, OR: 3.5) and DQB1*03 (41.2% *vs* 24.4%, *Pc* < 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 (*Pc* < 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 (*Pc* = 0.014, *Pc* < 0.001, and *Pc* < 0.001 respectively), while HLA-DQB1*060101 was negatively associated (*Pc* < 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 frequency of 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 (*Pc* = 0.01, *Pc* < 0.001, *Pc* < 0.001, and *Pc* = 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).

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

Characteristic	*n* (%)
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 ± 15.55
Microalbuminuria	
No	80 (94.1)
Yes	5 (5.9)
Frequency of DKA	1.22 ± 0.91
C-peptide (fasting)	0.34 ± 0.28
C-peptide (post-prandial)	0.58 ± 0.64
Serum cholesterol	163.95 ± 28.1
HbA1c	7.98 ± 1.59
Grades of HbA1c control[1]	
Optimal/suboptimal	57 (67.1)
Poor	28 (32.9)

[1]Optimal/suboptimal control < 8.5, poor control ⩾ 8.5. DKA: Diabetic ketoacidosis.

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 β cell[31,33].

T1D is considered a multifactorial condition with complex interactions between genetic and environmental factors[29,31]. There is evidence showing that 40%-50% of the inherited susceptibility to the disease is contributed by HLA-DR-DQ[30]. 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 (*Pc* < 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 (*Pc* = 0.014, *Pc* < 0.001, and *Pc* < 0.001 respectively), and HLA-DQB1*060101 was negatively associated (*Pc* < 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 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 (17.6)	2.21	1.35-3.62	0.001	0.014
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)	-	-	-	
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	0.6-49.5	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 genotype[1] 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
02/03	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
02/05	6 (7.1)	4 (3.5)	2.07	0.56-7.57	0.26	NS
02/06	6 (7.1)	16 (14.2)	0.46	0.17-1.23	0.16	NS
03/03	12 (14.1)	6 (5.3)	2.93	1.05-8.16	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	< 0.00	< 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

[1]Genotypes 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 genotype[1] 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 (0.45-2.78)	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

[1]Genotypes 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*0201 and DQB1*0302 were positively associated with T1D in various ethnic populations including Asians[34,35], European[36-43], and Americans[44-46]. Similar results were reported for Arab patients from Saudi Arabia[47,48], Kuwait[49], Tunisia[50], Lebanon[51], and Israeli[52]. On the other hand, DQB1*0301 and DQB1*0601 were negatively associated with T1D in Korean[34], Latin American[46], Lebanese[51], Tunisian[50], Saudi children[47,48], Turkey[43], and Romanian[37] populations.

In the literature, there is only one previous study investigating the association of HLA-DQB1 alleles with T1D in Egyptians. Gaber *et al*[53] 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 al*[53], 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 al*[53].

Egyptian are known to be of mixed ethnic origin (Middle Eastern, African and European)[54], so Egyptian studies are expected to add to the data available for different ethnic background[55]. 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[56]. 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 ($Pc = 0.01$, $Pc < 0.001$, $Pc < 0.001$, and $Pc = 0.04$, 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[44], Hungary[38], Romania[37], and Saudi Arabia[47,48].

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[57]. 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[58].

Geo-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[32]. Our results showed no correlation between HLA-DQB1 and diabetic nephropathy as the number of patients with microalbuminuria was considered as a limiting factor. Rønningen *et al*[59] 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 groups 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*02 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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