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Original Article

HLA-DRB, -DQA, and DQB alleles and haplotypes in Iranian patients with diabetes mellitus type I

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Specific alleles at the HLA-DRB1, -DQA1, and -DQB1 loci seem to be associated with variable risks of developing type 1 diabetes (T1D). This study assessed the distribution of HLA-DR and -DQ alleles among Iranian T1D patients and healthy controls. In this study, HLA-DRB1, -DQA1, and -DQB1 alleles were determined in 100 children with T1D and 100 unrelated healthy controls. The following alleles were found to have a strong positive association with T1D: DRB1*0301, DRB1*0401, DRB1*0402, DQA1*0301, DQA1*0501, DQB1*0201, and DQB1*0302. Meanwhile, protective associations were found for DRB1*1001, DRB1*1101, DRB1*15, DRB1*16, DQA1*0102, DQA1*0103, DQB1*0301, DQB1*0501, and DQB1*0602 alleles. The haplotypes found most frequently among patients with T1D were DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302, and DRB1*0402-DQA1*0301-DQB1*0302, whereas DRB1*1101-DQA1*0501-DQB1*0301 and DRB1*16-DQA1*0102-DQB1*0501 haplotypes were negatively associated with the disease. These results confirm the previously reported association of specific HLA-DR and HLA-DQ alleles and haplotypes with T1D in Iranian population. The notable difference was the identification of DRB1*16-DQA1*0102-DQB1*0501 as a protective haplotype and the absence of a negative association of DRB1*1301-DQA1*0103-DRB1*0603 with T1D.

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Diabetes mellitus type 1 (insulin-dependent) is a polygenic autoimmune disease resulting from progressive T-cell-mediated destruction of insulin producing pancreatic β islet cells, leading to dysregulation of glucose metabolism (1). Although the precise etiology of the disease remains elusive, the concordance rates of type 1 diabetes (T1D) in monozygote and dizygote twins could suggest that

genetic factors, in addition to environmental factors, are involved in the pathogenesis of T1D (2, 3). It is estimated that more than half of genetic susceptibility to T1D can be attributed to allelic variation in the human leukocyte antigen (HLA) region (2, 4). Linkage studies demonstrated that specific alleles at the DRB1, DQA1, and DQB1 loci are associated with a greater risk of developing T1D (5–8). A number of studies demonstrated that there are both predisposing and protective alleles at DR and DQ loci (9, 10). HLA DQB1*0201 and/or HLA DQB1*0302 are almost universally found to be associated with T1D, while the strongest negative association was observed for -DRB1*15011 -DQA1*0102 -DQB1*0602 haplotype (7, 11, 12). The type of association between particular HLA haplotype and disease may vary in various ethnic groups due to the linkage disequilibrium within the HLA class II region in the population studied (8, 9). In the Caucasian population, the higher risk of developing T1D was associated with DRB1*030101 -DQB1*0201 and DRB1*040101 -DQB1*0302 haplotypes, compared to DRB1*0405-DQB1*0401 and DRB1*0901-DQB1*0303 haplotypes in Japanese (13). Racial and ethnic diversity highlights the importance of HLA association studies in T1D in different populations. This study was performed to investigate the HLA class II (DRB, DQA1, and DQB1) allele and haplotype frequencies among Iranian children with T1D.

Materials and methods

Participants

The study population comprised 100 unrelated patients with T1D and the same number of non-diabetic healthy controls with normal fasting and random plasma glucose levels and no family history of T1D or other autoimmune disorders. The male/female ratio in the patient group was 0.64, compared to 0.58 in the control group. The mean age of the patients at the time of study was 13.9 ± 5.25 yr. Patients were selected randomly from those attending the Children's Medical Center Hospital, the pediatrics Center of Excellence in Tehran, Iran, from 2004 to 2008. Healthy controls were also randomly selected from unrelated healthy blood donors and matched by age and sex. T1D was defined according to the criteria of the American Diabetes Association (14). The classic symptoms of polydipsia, polyuria, and weight loss and the development of diabetic ketoacidosis were the most frequent findings at presentation. All patients were diagnosed before the age of 15 yr and required insulin for glycemic control. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and Health Services.

DNA extraction and HLA typing

Genomic DNA was extracted from peripheral blood lymphocytes by a modified salting out method (15). HLA-DRB, -DQA1, and -DQB1 typing was performed using polymerase chain reaction based on sequence-specific primers (PCR-SSP), as previously described (16, 17). The PCR reactions were carried out in 10 μL volumes. Samples were amplified in Techne genius thermal cyclers (St. Louis, MO, USA), after initial denaturation at 94°C for 2 min, followed by 10 cycles of 94°C denaturation for 10 s, 65°C annealing and extension for 60 s, and finally, 20 cycles of 94°C denaturation for 10 s, 61°C annealing for 50 s, and 72°C extension for 30 s. After amplification, PCR products were run on an agarose gel, and then the gel was interpreted for specific bands using a UV trans-illuminator. The haplotypes were calculated according to Iranian population-specific linkage disequilibrium pattern among HLA-DRB1, -DQA1, and -DQB1 alleles (18).

Statistical analyses

The differences in the HLA-DRB, -DQA1, and -DQB1 allele and haplotype frequencies between the studied groups were analyzed using the chi-square test with Yates's correction. Fisher's exact test was used when the minimum expected count was less than five. The odds ratio (OR) and 95% confidence interval (CI) of odds ratio were calculated. A p value of 0.05 or less was considered to be significant.

Results

DRB allele frequencies

DRB allele frequencies in patients with T1D and control subjects are shown in Table 1. Among the DRB1 alleles, DRB1*0301, DRB1*0401, and DRB1*0402 conferred the highest risks with the respective frequency of 39.5, 13.5, and 11.5% in the patients, compared to 10, 2.5, and 2.5% in the controls ($p < 0.001$). The OR were 5.88 (CI: 3.42–10.10), 6.07 (CI: 2.29–16.15), and 5.08 (CI: 1.89–13.62), respectively. The frequency of the following alleles decreased significantly in patients compared to controls: DRB1*1001 (0.5 vs. 4%, $p = 0.037$, OR: 0.12, CI: 0.01–0.97), DRB1*1101 (10 vs. 28.5%, $p < 0.001$, OR: 0.28, CI: 0.16–0.48), DRB1*15 (1 vs. 12%, $p < 0.001$, OR: 0.07, CI: 0.02–0.32), and DRB1*16 (1.5 vs. 6.5%, $p = 0.019$, OR: 0.22, CI: 0.06–0.78).

DQA1 and DQB1 allele frequencies

DQA1 allele frequencies in patients with T1D and control subjects are shown in Table 2. In the DQA1 region, the most frequent alleles in patients compared

Table 1. HLA-DRB allele frequencies in the patients with T1D and the controls

	T1D (N = 100)		Control (N = 100)			
DRB1	No.	%	No.	%	OR (95% CI)	p-Value
0101	5	2.5	11	5.5	0.44 (0.15–1.29)	0.201
0301	**79**	**39.5**	**20**	**10**	**5.88 (3.42–10.10)**	**<0.001**
0401	**27**	**13.5**	**5**	**2.5**	**6.09 (2.29–16.15)**	**<0.001**
0402	**23**	**11.5**	**5**	**2.5**	**5.07 (1.89–13.62)**	**0.001**
0403	1	0.5	3	1.5	0.33 (0.03–3.20)	0.623
0404	1	0.5	1	0.5	1.00 (0.06–16.10)	1.000
0405	10	5	5	2.5	2.05 (0.69–6.12)	0.292
0408	2	1	2	1	1.00 (0.14–7.17)	1.000
0701	9	4.5	13	6.5	0.68 (0.28–1.62)	0.511
0801	2	1	3	1.5	0.66 (0.11–4.01)	0.686
0901	7	3.5	6	3	1.17 (0.39–3.55)	1.000
1001	**1**	**0.5**	**8**	**4**	**0.12 (0.01–0.97)**	**0.037**
1101	**20**	**10**	**57**	**28.5**	**0.28 (0.16–0.48)**	**<0.001**
1301	6	3	9	4.5	0.66 (0.23–1.88)	0.600
1302	1	0.5	4	2	0.25 (0.03–2.22)	0.372
1401	0	0	11	5.5	–	–
1402	1	0.5	0	0	–	–
15	**2**	**1**	**24**	**12**	**0.07 (0.02–0.32)**	**<0.001**
16	**3**	**1.5**	**13**	**6.5**	**0.22 (0.06–0.78)**	**0.019**

HLA, human leukocyte antigen; T1D, type 1 diabetes; CI, confidence interval; OR, odds ratio.
The bolded values are significant at level of <0.05.

Table 2. HLA-DQA1 allele frequencies in the patients with T1D and the controls

	T1D (N = 100)		Control (N = 100)			
DQA1	No.	%	No.	%	OR (95% CI)	p-Value
0101	6	3	11	5.5	0.53 (0.19–1.47)	0.322
0102	**7**	**3.5**	**29**	**14.5**	**0.21 (0.09–0.50)**	**<0.001**
0103	**5**	**2.5**	**21**	**10.5**	**0.22 (0.08–0.59)**	**0.002**
0104	0	0	19	9.5	–	–
0201	8	4	15	7.5	0.51 (0.21–1.24)	0.197
0301	**72**	**36**	**26**	**13**	**3.76 (2.28–6.23)**	**<0.001**
0401	2	1	1	0.5	2.01 (0.18–22.35)	1.000
0501	**100**	**50**	**78**	**39**	**1.56 (1.05–2.33)**	**0.034**

HLA, human leukocyte antigen; T1D, type 1 diabetes; CI, confidence interval; OR, odds ratio.
The bolded values are significant at level of <0.05.

to controls were DQA1*0301 (36 vs. 13%, $p < 0.001$, OR: 3.76, CI: 2.28–6.23) and DQA1*0501 (50 vs. 39%, $p = 0.034$, OR: 1.56, CI: 1.05–2.33). The frequency of the following alleles decreased significantly in patients compared to controls: DQA1*0102 (3.5 vs. 14.5%, $p < 0.001$, OR: 0.21, CI: 0.09–0.50) and DQA1*0103 (2.5 vs. 10.5%, $p = 0.002$, OR: 0.22, CI: 0.08–0.59).

DQB1 allele frequencies in patients with T1D and control subjects are shown in Table 3. The most frequent alleles in the DQB1 region in patients compared to controls were DQB1*0201 (46.5 vs. 19%, $p < 0.001$) and DQB1*0302 (31 vs. 5.5%, $p < 0.001$). The OR were 3.70 (CI: 2.36–5.81) and 7.72 (CI: 3.92–15.20), respectively. In the DQB1 region, three alleles decreased significantly in patients compared to controls, including DQB1*0301 (11 vs. 31%, $p < 0.001$, OR: 0.27, CI: 0.16–0.47), DQB1*0501 (5.5 vs. 22%, $p < 0.001$, OR: 0.21, CI: 0.10–0.41), and DQB1*0602 (3 vs. 8.5%, $p = 0.03$, OR: 0.33, CI: 0.13–0.86).

DRB and DQ haplotypes frequencies

Table 4 summarizes the frequencies of HLA DRB1-DQA1-DQB1 haplotypes. The most frequent haplotype found in patients compared to controls was DRB1*0301-DQA1*0501-DQB1*0201 (39.5% vs. 10%, $p < 0.001$, OR: 5.88, CI: 3.42–10.10), followed by DRB1*0401-DQA1*0301-DQB1*0302 ($p < 0.001$) and DRB1*0402-DQA1*0301-DQB1*0302 ($p = 0.002$). The following haplotypes were negatively associated with the disease: DRB1*1101-DQA1*0501-DQB1*0301 ($p < 0.001$) and DRB1*16-DQA1*0102-DQB1*0501 ($p = 0.019$).

Frequency of homozygous DRB1*03 in the patients was 17%, which was significantly higher than 1% in the controls ($p < 0.001$). Indeed homozygous DRB1*04 was also more frequent in the patient group than controls (9% vs. 1%, $p = 0.023$). Frequency of heterozygous DRB1*03/DRB1*04 in the patients with T1D was 30%, which was also significantly higher than 3% in

Table 3. HLA-DQB1 allele frequencies in the T1D patients and the controls

	T1D (N = 100)		Control (N = 100)			
DQB1	No.	%	No.	%	OR (95% CI)	p-Value
0201	**93**	**46.5**	**38**	**19**	**3.70 (2.36–5.81)**	**<0.001**
0301	**22**	**11**	**62**	**31**	**0.27 (0.16–0.47)**	**<0.001**
0302	**62**	**31**	**11**	**5.5**	**7.72 (3.92–15.20)**	**<0.001**
0303	2	1	8	4	0.24 (0.05–1.16)	0.105
0401	3	1.5	2	1	1.50 (0.25–9.12)	0.500
0501	**11**	**5.5**	**44**	**22**	**0.21 (0.10–0.41)**	**<0.001**
0601	0	0	14	7	–	–
0602	**6**	**3**	**17**	**8.5**	**0.33 (0.13–0.86)**	**0.030**
0604	1	0.5	4	2	0.25 (0.03–2.22)	0.372

HLA, human leukocyte antigen; T1D, type 1 diabetes; CI, confidence interval; OR, odds ratio.
The bolded values are significant at level of <0.05.

the control subjects ($p < 0.001$). It should be noted that heterozygous DRB1*03/DRB1*04 have a higher association with the disease than the single homozygous DRB1*03 ($p = 0.045$) and homozygous DRB1*04 ($p < 0.001$) in the patient group, but not in the controls.

Discussion

This study provides the first comprehensive report of the association of HLA class II alleles and haplotypes with T1D in the Iranian population, stating that the Iranian population does not grossly divert genetically from other geographically adjacent populations. There are a number of studies reporting this association in different ethnic groups around the world. Among various HLA class II haplotypes observed in our studied population, DRB1*0301-DQA1*0501-DQB1*0201 was the most frequent haplotype in patients compared to controls. Moreover, DRB1*0401-DQA1*0301-DQB1*0302 and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes also conferred strong susceptibility to T1D. These findings are reminiscent of earlier findings of an association of DRB1*0301-DQA1*0501-DQB1*0201 with T1D among Turkish (19), Italian (20), Slovenian (21), Korean (22), Jamaican (23), and Arabian populations (24, 25).

In contrast, DRB1*1101-DQA1*0501-DQB1*0301 and DRB1*16-DQA1*0102-DQB1*0501 haplotypes were negatively associated with T1D; the protective role for DRB1*1101-DQA1*0501-DQB1*0301 were previously described in Caucasians (4, 26) and non-Caucasians (13). The frequency of DQA1*0501 allele was significantly higher in T1D patients and, thus, can be considered as a high-risk susceptibility allele. However, when it was combined with protective alleles, i.e., DRB1*1101 and DQB1*0501, the combined genotypes afforded some protections against T1D, whereas its combination with susceptible alleles, i.e., DRB1*0301 and DQB1*0201, was positively associated with T1D. Thus, it appears that DQA1*0501 did not play a significant role in T1D pathogenesis and that its implication in protection or susceptibily to T1D may be explained by the presence of protective or susceptible DRB1 and DQB1 alleles. This is the first report implicating the protective role of DRB1*16-DQA1*0102-DQB1*0501 haplotype in T1D.

The strong association of DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302, and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes with T1D and the protective role of DRB1*1101-DQA1*0501-DQB1*0301 haplotype in the Iranian population is similar to that found among Turkish (19), Italian (20), and Slovenian (21) populations. The notable difference was the identification of the protective role of DRB1*16-DQA1*0102-DQB1*0501 haplotype and the absence of a negative association of DRB1*1301-DQA1*0103-DRB1*0603 haplotype with T1D, which points to the impact of race and ethnicity on the differential distributions of HLA class II alleles and haplotypes.

HLA class II DR and DQ complex bind and present the processed antigen fragments to T cells. In T1D, HLA class II presents β-cell-specific peptides as autoantigens to autoreactive T cells. The differential affinity of the peptide-binding site of the DR-DQ complex to β-cell-specific peptides confers susceptibility to or protection from disease. The strong positive association of DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302, and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes, as well as the negative association of DRB1*1101-DQA1*0501-DQB1*0301 and DRB1*16-DQA1*0102-DQB1*0501 haplotypes with T1D is possibly due to the difference in autoantigenic fragment affinity of each haplotypes, as was suggested previously (27). DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0401-DQA1*0301-DQB1*0302, and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes could have lower affinity to β islet cell peptides presented to autoreactive T cells, and molecules encoded by DRB1*1101-DQA1*0501-DQB1*0301 and DRB1*16-DQA1*0102-DQB1*0501 haplotypes

Table 4. HLA class II haplotype frequencies in the T1D patients and the controls

DRB1/DQA1/DQB1 haplotypes	T1D (N = 100)		Control (N = 100)		OR (95% CI)	p-Value
	No.	%	No.	%		
DRB1*0101/DQA1*0101/DQB1*0501	5	2.5	11	5.5	0.44 (0.15–1.29)	0.201
DRB1*0301/DQA1*0501/DQB1*0201	**79**	**39.5**	**20**	**10**	**5.88 (3.42–10.10)**	**<0.001**
DRB1*0401/DQA1*0301/DQB1*0302	**28**	**14**	**5**	**2.5**	**6.35 (2.40–16.80)**	**<0.001**
DRB1*0401/DQA1*0301/DQB1*0303	1	0.5	0	0	–	–
DRB1*0402/DQA1*0301/DQB1*0302	**21**	**10.5**	**5**	**2.5**	**4.57 (1.69–12.39)**	**0.002**
DRB1*0403/DQA1*0301/DQB1*0302	1	0.5	1	0.5	1.00 (0.06–16.10)	1.000
DRB1*0403/DQA1*0301/DQB1*0401	0	0	2	1	–	–
DRB1*0404/DQA1*0301/0302	1	0.5	0	0	–	–
DRB1*0404/DQA1*0301/DQB1*0401	0	0	1	0.5	–	–
DRB1*0405/DQA1*0301/DQB1*0201	4	2	4	2	1.00 (0.25–4.05)	1.000
DRB1*0405/ DQA1*0301/DQB1*0302	6	3	1	0.5	6.15 (0.73–51.60)	0.122
DRB1*0405/DQA1*0301/ DQB1*0401	1	0.5	0	0	–	–
DRB1*0408/DQA1*0301/DQB1*0301	0	0	2	1	–	–
DRB1*0408/DQA1*0301/DQB1*0302	2	1	0	0	–	–
DRB1*07 DQA1*01/0201/DQB1*0201	8	4	13	6.5	0.60 (0.24–1.48)	0.370
DRB1*0801/DQA1*0401/DQB1*0401	2	1	0	0	–	–
DRB1*0801/DQA1*0501/DQB1*0301	0	0	1	0.5	–	–
DRB1*0801/DQA1*0601/DQB1*0301	0	0	2	1	–	–
DRB1*0901/DQA1*0301/DQB1*0302	6	3	0	0	–	–
DRB1*0901/DQA1*0301/DQB1*0303	1	0.5	6	3	0.16 (0.02–1.36)	0.122
DRB1*1001/DQA1*0101/DQB1*0501	1	0.5	0	0	–	–
DRB1*1001/DQA1*0104/DQB1*0501	0	0	8	4	–	–
DRB1*1101/DQA1*0501/DQB1*0301	**20**	**10**	**57**	**28.5**	**0.28 (0.16–0.48)**	**<0.001**
DRB1*1301/DQA1*0102/DQB1*0501	1	0.5	0	0	–	–
DRB1*1301/DQA1*0102/DQB1*0602	1	0.5	0	0	–	–
DRB1*1301/DQA1*0103/DQB1*0602	4	2	9	4.5	0.43 (0.13–1.43)	0.259
DRB1*1302/ DQA1*0102/ DQB1*0604	1	0.5	4	2	0.25 (0.03–2.22)	0.372
DRB1*1401/DQA1*0104/DQB1*0501	0	0	11	5.5	–	–
DRB1*1402/DQA1*0501/DQB1*0301	1	0.5	0	0	–	–
DRB1*15/DQA1*0102/DQB1*0501	1	0.5	2	1	0.50 (0.04–5.53)	1.000
DRB1*15/DQA1*0102/DQB1*0601	0	0	2	1	–	–
DRB1*15/DQA1*0102/DQB1*0602	0	0	8	4	–	–
DRB1*15/DQA1*0103/DQB1*0601	0	0	12	6	–	–
DRB1*15/DQA1*0103/DQB1*0602	1	0.5	0	0	–	–
DRB1*16/DQA1*0102/DQB1*0501	**3**	**1.5**	**13**	**6.5**	**0.22 (0.06–0.78)**	**0.019**

HLA, human leukocyte antigen; T1D, type 1 diabetes; CI, confidence interval; OR, odds ratio.
The bolded values are significant at level of <0.05.

have higher affinity to these autoantigens; thereby, protective alleles may prevent binding and presentation of crucial epitopes by high-risk alleles (27–29).

The contribution of HLA to T1D must be evaluated with regard to ethnic background. These results confirm the association of specific HLA-DR and HLA-DQ alleles and haplotypes with T1D, which were previously observed in other populations, and revealed some distinctive haplotypes, which may underlie the characteristic differences that distinguish Iranian diabetics, form other populations. Further studies to determine the frequency of HLA DRB1- DQA1-DQB1 haplotypes in the Iranian islet autoantibody positive vs. islet autoantibody negative patients affected by Type 1 diabetes could be suggested.

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