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Short Communication

Evaluation of Association Between HLA Class II DR4–DQ8 Haplotype and Type I Diabetes Mellitus in Children of East Azerbaijan State of Iran

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Abstract

Purpose: Association between HLA-DR4–DQ8 haplotype and type 1 Diabetes Mellitus (DM-1A) was investigated in children of East Azerbaijan state of Iran because such an association has not been previously studied in this population.

Methods: HLA-typing was performed by polymerase chain reaction sequence-specific priming. For haplotype analysis, the logistic regression model was performed.

Results: Of the three investigated alleles, the frequency of DRB1*0401 was significantly higher among patients compared with that in healthy subjects (76.74% vs. 23.26%).

Conclusion: The findings of the current study are consistent with those of previous studies and show that DRB1*0401 is associated with DM-1A; the frequencies of the two other alleles were also higher among patients, although the differences were not statistically significant. Two haplotypes associated with these alleles were also surveyed, and DRB1*0401−-DQA1*0301−, and DRB1*0401−-DQA1*0301−-DQB1*0302− were the most frequent haplotypes among the patient group.

Introduction

Type 1 Diabetes Mellitus (DM-1A) is an organ-specific, autoimmune,common chronic disease of children and young adults.About eighteen regions of the genome, mapped to the loci IDDM1 to IDDM18, have been linked with DM-1A risk; of these, the human leukocyte antigen (HLA) region (identified as IDDM1) accounts for more than half of the genetic susceptibility to DM-1A.[1] HLA-DR molecules of the HLA complex are known to be associated with DM-1A, and are nowadays used in clinical practice for prediction of disease development.[2] These facts indicate a substantial role for HLA in theimmunopathogenic processes leading to disease development.[3]

HLA plays an important role in self/non-self recognition, and is comprised of the highly polymorphic class I, class II and class III loci.[4] Fine mapping of the regions in HLA class II suggests that the most importantgenes are DQB1 and DRB1. Alleles of the DQB1 gene are often associated with alleles of the DRB1 gene.The contribution of specific HLA haplotypes toward DM-1A susceptibility depends on the ethnic/racial background.[5] This is highlighted by the positive associations of the haplotypesDRB1*030101-DQB1*0201and DRB1*040101-DQB1*0302 among Caucasianswhile the haplotypeDRB1*1501-DQB1*0602 appeared to offer protection againstDM-1A all populations.[6,7] Since data for the haplotype DR4–DQ8 and alleles DRB1*0401, DQA1*0301, and DQB1*0302 is not available for the children of East Azerbaijanpopulation, the current study aims to evaluate the genetic association between this haplotype and DM-1A risk.

Materials and Methods

Subjects

Eighty unrelated DM-1A patients were recruited from northwest Iran. DM-1A was diagnosed by the consultant endocrinologist according to clinical features and results of laboratory tests. The allele frequency of the HLA class II DR4–DQ8 haplotype was determined in the DM-1A patients. In addition, 80 control subjects with normal fasting/random blood glucose levels and no family history of DM-1A or other autoimmune diseases were included in the study (Table 1).

Table 1. Characteristic of DM-1A patients and normal subjects

Total numbers	Patients (n*=80)	Controls (n=80)
Gender	Females: 39(51.32%) Males: 41(48.81%)	Females:37(48.68%) Males: 43(51.19%)
Mean age of years	10.33±2.37	10.17±1.65
Insulin dependent	All	None
Family history of DM-1A	None	None
Fasting blood sugar (mg/dl)	134±4	97±2

HLA genotyping

Total genomic DNA was extracted from the peripheral blood of study participants using the salting-out

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method, and used for polymerase chain reaction (PCR) analysis. PCR-Sequence-Specific Priming (PCR-SSP) technique was used for genotyping of DRB1*0401-DQB1*0302-DQA1*0301 haplotype, as describedby IonescuTirgoviste et al.[8] The PCR products were analyzed by agarose gel electrophoresis using a 1% (w/v) agarose gel, and visualized by staining with Simply Blue safe stain (Fermentas).

Data analysis

Data was analyzed using STATA11 statistical software package (STATA Corporation, Texas). For preliminary descriptive statistics, variant analyses were done using chi-square test and calculation of odd ratios (ORs) with 95% confidence interval (CI). For analyses of haplotype effects, the logistic regression modules developed for STATA were used. This procedure generated tabulated values detailing frequency probability of various alleles along with adjusted ORs for minor alleles compared to the reference major allele. P value and 95% CI were also reported. P value < 0.05 was considered as statistically significant. This study was approved by the Regional Committee of Ethics of the Tabriz University of Medical Sciences. Written informed consent was obtained from the parents of all the children who participated in this study.

Results

*Association of the alleles DRB1*0401, DQB1*0302, and DQA1*0301 with DM-1A*

Allele frequencies of HLA-DRB1*0401, DQA1*0301, and DQB1*0302 in DM-1A patients and healthy controls are shown in Table 2. The DRB1*0401 allele showed the highest frequencyamong patients compared to controls (76.74% vs. 23.26%);the other two alleles DQA1*0301(51.11% vs. 48.89%) and DQB1*0302(61.76% vs. 38.24%) also showed a similar trend. HLA-DRB1*0401 showed the strongest association with DM-1A (P=0.00, OR=4.91, 95% CI= 2.10–11.45). In addition, the frequencies of HLA-DQA1*0301 (P=0.86, OR=1.06, 95% CI=0.53–2.12) and DQB1*0302 (P=0.12, OR=1.83, 95% CI=0.83–4.01) were also higher in patients than controls, albeit the differences were not statistically significant. Surprisingly, DRB1*0401 was the only allele that showed significantly positive association with DM-1Aamong this data set.

Table 2. HLA allele distribution in subjects with DM-1A

	DM- 1A	Control	P value	OR*	95%CI#
	n (%)	n (%)			
DRB1*0401	+:33(76.74) -:47(40.17)	+:10(23.26) -:70(59.83)	0.00	4.91	2.10-11.45
DQA1*0301	+:23(51.1) -:57(49.57)	+:22(48.89) -:58(50.43)	0.8	1.06	0.53-2.12
DQB1*0302	+:21(61.76) -:59(46.83)	+:13(38.24) -:67(53.17)	0.1	1.83	0.83-4.01

*Odds Ratio #Confidence interval

*Association of the haplotype DRB1*0401-DQB1*0302 with DM-1A*

The association of DRB1*0401-DQB1*0302 haplotype with DM-1A was investigated, and four haplotype frequencies were calculated(Table 3). The most frequent haplotype, DRB1*0401^{-}-DQB1*0302^{-}, was used as a reference haplotype for the calculation of ORs. As presented in this table,the haplotypes DRB1*0401^{-}-DQB1*0302^{+} and DRB1*0401^{+}-DQB1*0302^{-} were frequently associated with the patient group, with the DRB1*0401^{+}-DQB1*0302^{-} haplotype showing significant positive association with DM-1A (OR=6.25, 95%CI=2.51±16.96). The frequencies of the various haplotypes are shown in Table 3.

Table 3. DRB1*0401-DQB1*0302 frequency and haplotype associations with DM-1A

Haplotypes	Frequency	OR	P>|z|	95%CI
DRB1*0401^{-}-DQB1*0302^{-}	0.88	-	-	-
DRB1*0401^{-}-DQB1*0302^{+}	0.05	2.77	0.03	1.09±7.03
DRB1*0401^{+}-DQB1*0302^{-}	0.04	6.52	0.00	2.51±16.96
DRB1*0401^{+}-DQB1*0302^{+}	0.02	0.38	0.8	0.00±453

*Association of the haplotype DRB1*0401-DQB1*0302-DQA1*0301 with DM-1A*

The association of the DRB1*0401-DQB1*0302-DQA1*0301haplotype with DM-1A was investigated, and five haplotype frequencies were calculated (Table 4). The most frequent haplotype (DRB1*0401^{-}-DQB1*0302^{-}-DQA1*0301^{-}) was used as a reference haplotype for the calculation of ORs.Among these haplotypes, DRB1*0401^{+}-DQB1*0302^{-}-DQA1*0301^{-} haplotype was significantly associated with DM-1A (OR=6.68, 95%CI=2.68±16.61). The haplotype associations and related ORsare reported in Table 4.

Table 4. DRB1*0401-DQB1*0302-DQA1*0301 frequency and haplotype associations with DM-1A

Haplotypes	Frequency	OR	P > |z|	95%CI
DRB1*0401^{-}-DQB1*0302^{-}DQA1*0301^{-}	0.74	-	-	-
DRB1*0401^{-}-DQB1*0302^{-}DQA1*0301^{+}	0.13	2.91	0.01	1.27±6.65
DRB1*0401^{-}-DQB1*0302^{+}DQA1*0301^{-}	0.05	6.68	0.00	2.68±16.61
DRB1*0401^{+}-DQB1*0302^{-}DQA1*0301^{-}	0.04	4.40	0.98	0.00±.
DRB1*0401^{+}-DQB1*0302^{+}-DQA1*0301^{-}	0.02	OR	P > |z|	95%CI

Discussion

The risk of developing autoimmune diseases,in which immune cells destroy healthy cells in the body, is sometimes related to the alleles of HLA class II genes in the body.[9] Previous studies showed that inheriting certain alleles of the HLA class II genes increases the probability that immune cells will destroy the body's healthy cells.[10] The identity of these HLA genes is unclear because certain variants appear to be present only on particular haplotypes, which may also be population-specific.[11] Different HLA haplotypeshave different effects on susceptibility to DM-1A, depending on the population under study.[12] For example, certain HLA allelesincreasethe risk of DM-1A among Caucasians,while others likeDRB1*1501-DQB1*0602appear to provide protection againstDM-1A in all populations. The current study evaluatedthe frequencies of DRB1*0401, DQA1*0301,and DQB1*0302alleles and their haplotypes inDM-1A patients and normal controls to assess the contribution of HLA class II genes to DM-1A susceptibility. The findings of this study are consistent withthose of previous studies and show that DRB1*0401 is associated with DM-1A; the frequencies of the two other alleles were also higher among patients,although the differences were not statistically significant. HLA genes are not the only ones involved in DM-1Asusceptibility;therefore, future studies should be focused on the identification of other loci that affect DM-1A and elucidate their association. The current study is the first in this population to investigate the influence of DR4–DQ8 haplotype on DM-1A with complete molecular genotyping;the allele and haplotype frequencies of HLA-DRB1*0401, -DQA1*0301, and -DQB1*0302 have been presented, which can also prove useful as basic data for anthropology and disease association studies.

While considering the impact of the findings in the current study, important limitations must also be regarded. First, this study was a single-center case-controlled study, and subsequent investigations employing a larger number of patients and controls would be useful for confirming the role of these alleles and their haplotypes in DM-1A risk. Second, the association between the DR4–DQ8 haplotype and DM-1A was observed among subjects from the northwest of Iran. Further studies are required to clarify this association among populations in different regions of Iran. Based on these observations, it can be constructed that HLA-DRB1*0401 as a single factor has a very modest effect with respect to the risk for DM-1A. Further studies addressing the association of other polymorphic genes located at the HLA loci with DM-1A susceptibility is required.

Conclusion

The findings of the current study are consistent with those of previous studies and show that of DRB1*0401 allele was associated significantly with DM-1A. Meanwhile the frequency of DRB1*0401−-DQA1*0301−, and DRB1*0401−-DQA1*0301−-DQB1*0302 haplotypes were higher among the patients group.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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