The association between HLA DQ genetic polymorphism and type 1 diabetes in a case-parent study conducted in an admixed population

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Abstract. Susceptibility to the type 1 diabetes is genetically controlled and there is an increased risk associated with the presence of some specific alleles of the human leukocyte antigens class II loci (DQA1 and DQB1 genes). The purpose of this study is to evaluate the association between type 1 diabetes and HLA DQ alleles using case-parents trios in the admixed population of Uruguay composed by a mixture of Caucasian, Amerindian and Negroid populations. DQA1 and DQB1 genotyping was performed by polimerase chain reaction followed by oligospecific probes hybridization in 51 case-parents trios. The transmission disequilibrium test was used for detecting differential transmission in the HLA DQ loci. DQB1*0302 was the only allele for which preferential transmission is suggested (probability of transmission = 67.56%; exact p-value TDT = 0.047 uncorrected for multiple comparisons). DQA1*0301 allele showed a trend for preferential transmission without achieving statistical significance. This result would confirm the hypothesis previously advanced in a case-control study. Therefore, DQB1*0302 allele could be considered as the most important susceptibility allele for developing type 1 diabetes in Uruguay population.

Key words: HLA, IDDM, (TDT), Type 1 Diabetes Mellitus, Uruguay

Type 1 diabetes mellitus is an autoimmune disorder with a complex etiology including genetic susceptibility mainly conferred by the HLA complex [1]. Diabetes mellitus is a frequent illness in Uruguay, occurring with an estimated frequency of 6/100 [2]. Although no definitive numbers were established, it may be estimated that 10% of the affected individuals would correspond to type 1 diabetes. Susceptibility to the development of type 1 diabetes has been correlated with specific alleles of HLA DQ loci (DQA1 and DQB1). The observed frequencies of such alleles vary in relation to geographical zones and to the ethnic origin of each population [3]. The Uruguayan population is the result of an intense process of miscegenation of three ethnic groups, Caucasian, Amerindian and Negroid [4, 5]. The aim of this study is to evaluate the association between type 1 diabetes and HLA DQ alleles using the case-parents design in the Uruguay admixed population.

A total of 51 affected children with type 1 diabetes (25 females and 26 males) and their parents were selected for this study. The following criteria were considered to include patients in this study: (1) age under 15 years old, (2) the possibility of studying both parents, (3) the diabetic patients were considered as affected of type 1 diabetes according to the American Diabetes Association criteria. The cases attended public and private health centers and all the participants were informed of this study, and the parents agreed to participate by a written consent.

Genomic DNA was extracted from peripheral blood using DNAzol (Promega). Cases and parents were genotyped for HLA DQ genes using polymerase chain reaction and dot-blot hybridization [6]. Allelic variants DQB1*0501 and *0502 are genotyped as DQB1*0501. Alleles DQB1*0601 and *0602 are genotyped as DQB1*0602. The primers and probes used for this study were defined in the 11th and 12th International Workshop of Histocompatibility [7, 8]. For each allele, the official nomenclature of the World Health Organization Committee for factors of the HLA was used [9]. Transmission-disequilibrium test (TDT) was used to assess the differential pattern of excess transmission of HLA alleles from heterozygous parents to diseased children [10]. By sampling family trios through the affected child (probands), the associations between HLA alleles and type 1 diabetes

Table 1. Parental genotypes (n = 102)

	N	%
DQA1* locus		
*0301/X[a]	5	4.90
*0501/X	17	16.67
*0301/*0501	23	22.55
*0301/*0301	30	29.41
*0501/*0501	26	25.49
X/X	1	0.98
DQB1* locus		
*0201/X[b]	25	24.51
*0302/X	19	18.63
*0201/*0302	18	17.65
*0201/*0201	23	22.55
*0302/*0302	12	11.76
X/X	5	4.90

[a]X = other than *0301 or *0501.
[b]X = other than *0201 or *0302.

Table 2. TDT results for HLA-DQ alleles and type 1 diabetes in 51 case-parent trios

	Heterozygous parents	Transmitted/ non-transmitted	*p*-Value
DQA1* locus			
*0101	6	1/5	0.22
*0102	3	1/2	1.00
*0103	10	5/5	1.00
*0201	3	0/3	0.25
*0301	28	17/11	0.34
*0501	40	21/19	0.87
DQB1* locus			
*0201	43	24/19	0.54
*0301	22	8/14	0.29
*0302	37	25/12	0.047
*0303	3	0/3	0.25
*0501	8	1/7	0.07
*0503	1	0/1	1.00
*0602	3	1/2	1.00
*0603	11	5/6	1.00

Exact *p*-values uncorrected for multiple comparisons.

would cause transmission to appear different from the expected value (probability of transmission = 0.5). TDT represents a valid test of association even if population stratification is present [10]. Initially, multiple-allele versions of the TDT were performed to assess global association between HLA DQ alleles and type 1 diabetes. Additionally, allele-specific uncorrected *p*-values were calculated for each allele. Given the modest sample size and data sparseness in transmission tables, global and allele-specific *p*-values for association using TDT were computed through exact methods implemented in the statistical package STATA 7.0 (Stata Coporation, College Station, TX, 2001) [11, 12].

The parental haplotypes are shown in Table 1. The estimated frequency of heterozygous genotypes in the parental genotypes was 45 (44.12%) for DQA1* and 64 (62.74%) for DQB1* loci (Table 1).

The TDT global exact *p*-value for gene-disease associations was calculated as 0.23 for DQA1 gene and 0.25 for DQB1 locus. From the 45 heterozygous parental genotypes, 40 of them carried the DQA1*0501 allele (88.89%), and 21 individuals transmitted it (probability of transmission = 52.50%). Regarding DQA1*0301 allele, a total of 17 transmissions occurred from 28 heterozygous parents carrying such allele (probability of transmission = 60.71%) (Table 2). The alleles *0101, *0102, *0103, *0201 were transmitted, in each case with a frequency equal or lower than 50%. The allele DQA1*0402 was not present in the parents.

From the 64 heterozygous parental genotypes, the DQB1*0201 allele was present in 43 cases (67.19%) and 24 of them transmitted it (probability of transmission = 55.81%). DQB1*0302 alleles was present as a heterozygous combination in 37 (57.81%) of individuals and 25 of them transmitted it to the affected cases (probability of transmission = 67.57%) (Table 2). The remaining analyzed alleles were present in frequencies lower than 50%.

In the 51 probands the most frequent genotypes were: DQA1*0301−DQB1*0302/DQA1*0501 − DQB1*0201 (29.41%) and DQA1*0501/DQB1*0201 −DQA1*0501/DQB1*0201 (21.57%) and the haplotypes DQA1*0301−DQB1*0302 (36.27%) and DQA1*0501−DQB1*0201 (45.10%) were the most commonly found.

The transmission of these haplotypes from heterozygous parents to affected siblings was analyzed assuming an HLA DQ haplotype reconstruction based on the most commonly linkage disequilibrium published for these loci. An excess of transmission for DQA1*0301 −DQB1*0302 haplotype (67.56%). was found in affected children (25 transmitted haplotypes vs. 12 non-transmitted; probability of transmission = 67.56%; *p*-value exact TDT = 0.047). As to the DQA1*0501 −DQB1*0201 haplotype, 42 parents were heterozygous for this haplotype and it was transmitted in 24 occasions (probability of transmission = 57.14%), although no statistically significant differences in preferential transmission were observed (*p*-value exact TDT = 0.54).

The population of Uruguay constitutes a melting pot of populations (Caucasian, Negroid and Indoamerican). These groups have undergone a particular and intense process of admixture in such a way that today there are no isolated Amerindian groups to be found in Uruguay. Family-based association studies such as the case-parent design, allow analysis of inherited vs. non-inherited alleles among disease subjects, thus eliminating the confounding effect

due of population stratification by ethnicity that may generate spurious associations in case-control studies [10]. The global test for association was not significant either for DQA1 or DQB1 genes. However, our results showed that some HLA DQ alleles were transmitted from parents to the affected cases with a frequency greater than 50%. In this context, DQB1*0302 was the only allele for which preferential transmission is suggested (probability of transmission = 67.56%; exact p-value TDT = 0.047, uncorrected for multiple comparisons). This result would confirm the hypothesis advanced in a previous case-control study [5]. Therefore, DQB1*0302 allele could be considered as a susceptibility allele to type 1 diabetes in our population. Although non-significant association was found in multiple-allele TDT, this result is probably the consequence of our modest sample size and the limited number of heterozygous parents examined.

DQB1*0201 has not been consistently shown to be a susceptibility allele in other populations [8]. In our previous case-control study, this allele was not statistically associated to the disease either [5]. In the present study, this allele presents a preferential transmission (55.8%) but no statistical significance.

Our Caucasian population comes from a variety of backgrounds, it is mainly composed of people who emigrated from Spain, Italy and France [13]. Only in southern Europe, the DQB1*0201 allele can be considered as a high-risk allele for type 1 diabetes [14, 15].

Up until now, no information about DQA frequencies in Uruguayan population has been published. We found that allele DQB1*0302 and, at a lower extent, DQA1*0301 were preferentially transmitted from heterozygous parents to affected children. Due to our limited number of heterozygous parents and the large number of alleles in HLA loci, global TDT for multiple alleles and statistical corrections for multiple comparisons do only allow to establish suggestive association between DQB1*0302 allele and type 1 diabetes (p-value = 0.047). DQA1*0301 allele showed a trend for preferential transmission without achieving statistical significance. It is interesting to emphasize this result because this allele is consistently assumed to be a susceptibility allele in population-based case-control studies conducted in countries with ethnic backgrounds that are varied [8].

In our study, alleles DQA1*0101-*0102-*0103-*0201, DQB1*0303-*0501-*0602-*0603-*0503 appear to be inversely associated with type 1 diabetes, it is also possible to hypothesize that such allelic variants are neutral and they are not transmitted when paired with a high risk allele such as DQA1*0301 or DQB1*0302. In conclusion, our results suggest that DQB1*0302 could be considered as the most important susceptibility allele for developing type 1 diabetes in Uruguay population.

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