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Association between diabetes type 1 and DQB1* alleles in a case-control study conducted in Montevideo, Uruguay

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ABSTRACT. We studied HLA DQB1 allele frequencies and the relative risk (RR) of various genotypes in 72 type 1 diabetic patients and 40 control individuals in Uruguay. This is a tri-racial (Caucasian, Black and Indo-American) mixed population. The products of the polymerase chain reaction amplifications were hybridized with oligonucleotides by allele-specific oligonucleotide reverse or dot blot methods. Significant differences between these two groups were observed only for allele DQB1*0302 (35%, RR = 7.34, P<0.001). The frequency of the alleles carrying a non-aspartic acid residue at position 57 was significantly higher in the diabetic patients (85 *vs* 53%, P<0.001). In contrast, the frequency of Asp alleles was negatively associated with type 1 diabetes (RR = 0.20, P<0.001). The genotype DQB1*0302/DQB1*0201 (33%, RR = 5.41,

P<0.05) was positively associated with this disease. The genotype frequencies associated with type 1 diabetes in our population were significantly different from what is known for Caucasian and Black populations as well as compared with another admixed population, from Chile.

Key Words: Diabetes type 1, Human leukocyte antigen system, HLA

INTRODUCTION

Insulin-dependent diabetes mellitus (type 1) is a chronic autoimmune disease with a subclinical prodromal stage characterized by selective destruction of insulin-producing beta cells in the pancreatic islets (Knip, 1997). Autoimmune diabetes is the clinical manifestation of a sequential cascade of immunological events that occur in a genetically susceptible individual. Structural and functional analysis of the human leukocyte antigen system (HLA) class II susceptibility genes in type 1diabetes suggests the participation of molecular mechanisms in several of the key steps in this cascade of autoimmune events. A pathway has been outlined in which the HLA-DQ genes associated with this disease would bias the immunology repertoire towards autoimmune specificities, creating an autoimmune-prone individual, followed by amplification and triggering events that promote subsequent immune activation (Nepom and Kwok, 1998). It is now obvious that the genetic background of an individual, poorly characterized environmental factors and aggressive autoimmune phenomena are interrelated in their contribution to the clinical progression of this type of diabetes. At present only 60 to 65% of the genes that determine susceptibility to type 1 diabetes are known. Such genes are associated either with the HLA region on chromosome 6p21 or the insulin locus on chromosome 11p15. At the moment, at least eight additional susceptibility genes are under investigation (Morwessel, 1998). The effects of most diabetes-predisposing genes are weak, with the exception of HLA region susceptibility genes (Field, 2002). Specifically, HLA-DQ and DR are considered as the most important, contributing to 48% to the development of this illness, and they are closely associated with susceptibility and resistance (Verge and Eisenbarth, 1996; Gorodezky et al., 1997; Ronningen, 1997; Schranz and Lernmark, 1998; Dahlquist, 1998; Ettinger et al., 1998). There are many different combinations of these genes conferring distinct degrees of risk to this disease (Ronningen, 1997). The mechanisms that mediate the genetic impact may differ in different populations as well as in different ethnic groups and they also vary according to geographical region (Akerblom et al., 1997a,b).

The DIAMOND project tested the hypothesis that the geographical differences in the incidence of type 1 diabetes vary according to the frequency of certain susceptibility alleles in the HLA system (Dorman, 1997). Collaborative studies, as well as individual reports on populations, have shown the participation of these alleles in this illness (Todd et al., 1987; Palavecino et al., 1990; Erlich et al., 1993; Thorsby and Ronningen, 1993; Ronningen, 1997; Thomson et al., 1998; Caillat-Zucman et al., 1998); this variation depends on the ethnic origin or geographical location of the population. Therefore, it is important to determine which alleles are associated with type 1 diabetes mellitus in each population. There is no published information on the DQ alleles involved in this pathology for Uruguay.

The Uruguayan population is a mixture of populations originating from different parts of the world: Caucasian, Negroid and Indo-American. Caucasian people arrived mainly from

Spain, Italy, France, Portugal and Lebanon. Previous to the arrival of these immigrants, native groups mainly "charrúas", "minuanes", and "guaranies", among others, existed in our country. In the 17th and 18th centuries the region was colonized by Portugal and Spain, and shortly thereafter African slave trade began on a large scale. This group grew so rapidly that at the beginning of the 18th century it constituted a third of the population of the capital city (Montevideo). These Africans were Bantu from the coasts of the Congo and of Angola (Pi and Vidart, 1969; Sans and Barreto, 1998; Bonilla, 1998). Since then, there has been an intense admixture of these three groups in Uruguay. This fact differentiates our population from other Latin-American countries, since no isolated groups of Amerindians are found. The contribution of these three different groups to the population of Montevideo city was estimated in a recent paper (Sans et al., 1997) as: 92% Caucasian, 1% Amerindian and 7% Negroid.

Based on these data, the control group of our study for type 1 diabetes was taken from a DNA bank where individuals were selected from public and private health centers, taking into consideration socio-economic parameters. The relative frequencies of DQB1* alleles and relative risks for each haplotype were determined.

MATERIAL AND METHODS

Seventy-two patients (35 males and 37 females) with typical type 1 diabetes mellitus, according to World Health Organization (WHO) criteria, were included in our study. The mean age at diagnosis was 10.7 ± 6.4 years. The control group was 40 genetically unrelated individuals obtained as a random sample from a DNA bank comprising 500 individuals kept at the Cytogenetics Department, IIBCE. These DNA samples assure a correct representation of the general population, given that they were selected based on economic and social criteria from public and private health centers. Both groups live in the same area (Montevideo).

DNA was extracted from peripheral blood with the phenol-chloroform technique or with DNAzol (Promega). Exon 2 of the gene DQB1 (255 bp) was amplified by polymerase chain reaction, utilizing specific primers. The products were hybridized with oligonucleotides by one of the two techniques: 1) allele-specific oligonucleotide reverse (ASO reverse) and 2) dot blot (Pérez-Bravo et al., 1995). Primers and probes used for this study were defined in the 11th and 12th International Workshops of Histocompatibility (Caillat-Zucman et al., 1998). The official nomenclature of the HLA system of the Committee of the WHO was used in all cases. Allele frequencies were calculated from the genotype data by the gene count method. The relative risk (RR) was estimated as an odds ratio by the method of Wolf (with Haldane's correction). We also estimated the etiological fraction. A chi-square test (with Yates correction) was used to determine the statistical significance of all calculations. Statistical analyses were made by using the STATA 5.1 statistical software (StatSoft, 1995). Each individual participating in this study was previously informed and a written consent was obtained.

RESULTS

There were significant differences between DQB1* allele frequencies in type 1 diabetes patients and controls (Table 1). The frequency of the allele DQB1*0302 was higher in patients than in controls, with an RR of 7.34. On the other hand, allele DQB1*0402 was significantly less frequent in diabetes patients (RR = 0.10). We examined the frequency of amino acid residues at position 57 of the HLA-DQ β chain. DQB1-Asp^{57} (A) encoding genes (DQB1*0301, -0303,

-0402, -0602, -0603, -0607) and non-DQB1-Asp^{57} (NA) encoding genes (DQB1*0201, -0302, -0501, -0604) were found in the samples. The frequency of alleles lacking an aspartate residue at position 57 on the DQB1 chain (NA) was significantly higher in the type 1 patients than in the controls with an RR of 4.93. Among all the NA alleles, only DQB1*0302 was significantly associated with type 1. On the other hand, the frequency of Asp alleles (A) was negatively associated with type 1 (RR = 0.20, P<0.001).

Table 1. HLA DQB1 allele frequencies in diabetic patients and controls.

Alleles DQB1	Patients (%) (N = 144)	Controls (%) (N = 80)	Corrected P value
NA	85	53	<0.001
*0201	47	30	0.125
*0302	35	6	<0.001
*0501	3	13	0.133
*0604	0	4	0.421
A	15	48	<0.001
*0402	1	9	0.041
*0602	1	5	0.499
Others	14	34	0.006

NA: Asp^{57} negative, A: Asp^{57} positive. Others: A alleles nonsignificant.

The NA/NA genotypes were positively associated with disease (RR = 4.88, P<0.001) and the A/A genotypes were negatively associated with diabetes (RR = 0.04, P<0.001) (Table 2). Only one genotype, DQB1*0302/DQB1*0201 (33%, RR = 5.41), was present at significantly different frequencies in the comparison between the two groups. There was also a significant difference between the genotypes DQB1*0201/DQB1*0302 and non-DQB1*0201/DQB1*0302 (P<0.001). There were no significant differences between males and females.

Table 2. HLA DQB1 genotype frequencies among diabetic patient and control groups.

Genotype DQB1*	Patients (%) (N = 72)	Controls (%) (N = 40)	Corrected P value
NA/NA	71	33	0.0025
*0201/*0201	22	10	0.8882
*0201/*0302	33	8	0.0344
*0201/*0501	0	10	0.3094
*0302/*0302	11	0	0.4717
*0302/*0501	3	3	0.9999
*0501/*0501	1	3	0.9999
NA/A	28	40	0.9714
*0201/*0402	0	3	0.9999
*0201/X	15	20	0.9999
*0302/X	11	3	0.9332
X/X	1	15	0.1839
A/A	1	28	<0.001
*0402/*0402	0	5	0.9650
*0402/X	0	5	0.9650
X/X	1	18	0.0724

NA: Asp^{57} negative, A: Asp^{57} positive, X: Alleles other than *0201, *0302, *0402.

DISCUSSION

Considerable information has been published about the relationship between HLA genes and type 1 diabetes. Recently, Field (2002) stressed the importance of such susceptibility genes in the determination of insulin-dependent diabetes mellitus. Collaborative studies, such as the DIAMOND project and the DAISY study (Palavecino et al., 1990; Rewers et al., 1996; Dorman, 1997) have clearly demonstrated ethnic and geographical variants in HLA frequencies. Nevertheless, there have been few studies published on our admixed population in Uruguay.

There are specific alleles of the HLA DQB gene that are associated with susceptibility and protection factors for the development of the diabetes (Baisch et al., 1993). It has been observed that DQB1*0302 confers susceptibility in a dominant form while DQB1*0602 confers dominant protection (Erlich and Bugawan, 1989; Gutierrezlopez et al., 1992; Sorrentino et al., 1992; Kockum et al., 1993; Tosi et al., 1993; Forbes et al., 1993; Cavan et al., 1993). We found that the DQB1*0302 alleles are present in patients with type 1 diabetes at a significantly higher frequency than in the control group. Therefore, this allele is strongly associated with this disease in our population. The allele DQB1*0201 was also significantly associated. The heterozygous form DQB1*0201/DQB1*0302 was found to be highly associated with type 1 diabetes. When we compared this genotype with non-DQB1*0201/DQB1*0302 the difference in frequencies was significant. This genotype is associated with more aggressive or rapid disease development (Caillat-Zucman et al., 1998). There were significantly more protective alleles in the control group and various genotypes can differentiate the corresponding samples into two subgroups. Based on the test for homogeneity, these two groups behave as two different populations, with highly significant differences. Both groups (patients and controls) were found to be in Hardy-Weinberg equilibrium. We compared our results with publications concerning Caucasian, Black and Chilean populations (Ronningen et al., 1992; Santos et al., 2000) and found significant differences between our population and these three groups. When analyzing the genotype DQB1*0201/DQB1*0302, which was highly associated with diabetes type 1 in our population, only the Caucasian group was not significantly different. This makes sense, as this ethnic group is the main contributor to our present population (Sans et al., 1997). Nevertheless, differences among genotypes DQB1*0201/DQB1*0201, DQB1*0201/DQB1*X and DQB1*0302/DQB1*X were found between our patients and the Caucasian study. These results are apparently a consequence of the mixing process that occurred in our population. Given the association of type 1 diabetes with specific susceptibility alleles in a heterogeneous group, it is likely that these results reflect real susceptibility for this disease. These facts should allow us to improve genetic counseling for our population, concerning the risk of developing diabetes. This type of information would enable clinicians to use genetic information to predict which children are predisposed to this disease and to identify them as candidates for future preventive therapies (Field, 2002). These tendencies will be examined in a more extensive study with other HLA markers.

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