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Distribution of HLA-DRB1 alleles in a mixed population with insulin-dependent diabetes mellitus from the Southeast of Brazil

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Abstract

HLA class II genes are strongly associated with susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM). The present study reports the HLA-DRB1 genotyping of 41 IDDM patients and 99 healthy subjects from the Southeast of Brazil (Campinas region). Both groups consisted of an ethnic mixture of Caucasian, African Negro and Amerindian origin. HLA-DRB1*03 and *04 alleles were found at significantly higher frequencies among IDDM patients compared to the controls (DRB1*03: 48.8% *vs* 18.2%, P<0.005, RR = 4.27; DRB1*04: 43.9% *vs* 15.1%, P<0.008, RR = 4.37) and were associated with a susceptibility to the disease. DRB1*03/*04 heterozygosity conferred a strong IDDM risk (RR = 5.44). In contrast, the HLA-DRB1*11 allele frequency was lower among IDDM patients (7.3% *vs* 26.3% in controls), but the difference was not significant. These data agree with those described for other populations and allow genetic characterization of IDDM in Brazil.

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- Genetic markers
- DNA typing
- HLA-DRB1

Introduction

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune syndrome resulting from the destruction of pancreatic ß cells by autoreactive T lymphocytes (1,2). The development of the disease involves the interaction of multiple genes and environmental factors (1,2). Several studies have indicated a prominent role for major histocompatibility complex (MHC) genes on chromosome 6 in conferring susceptibility and resistance to IDDM in a variety of human populations (3-5). In particular, the class II HLA region that encodes molecules involved in antigen presentation to CD4+ T cells represents the strongest genetic marker for the disease (3-5). The association of IDDM with the HLA-DR specificities DR3 and DR4 was initially demonstrated by serological HLA analysis (6-8). HLA genotyping has now confirmed these data and has also facilitated the identification, in Caucasians, of the two most strongly predisposing haplotypes, the DRB1*03-DQA1*0501-DQB1*0201, and DRB1*04-DQA1*0301-DQB1*0302 (3).

The genetic basis of IDDM has been poorly investigated in the Brazilian popula-

tion. Only one report by Eizirik et al. (9) has described the frequency of serologically defined DR antigens in IDDM patients. The Brazilian population is characterized by a complex genetic background resulting from the intermixing of Caucasians, Negroes and Amerindians (10). The aim of the present study was to determine the distribution of HLA-DRB1* alleles in a mixed population of IDDM patients from Southeastern Brazil.

Material and Methods

Subjects

Forty-one unrelated IDDM patients diagnosed according to the National Diabetes Data Group criteria (11) and accompanied at the Endocrinology Service of the University Hospital of the State University of Campinas (UNICAMP) were studied. The group consisted of 26 women and 15 men. Mean age at the time of IDDM diagnosis was 17.7 ± 7.9 years. All of the patients were residents of Campinas and the surrounding region in the State of São Paulo, and had a mixed genetic background of European, African and Amerindian origin. Ninety-nine racially matched healthy subjects from the same geographic area were included as the control group. All subjects gave informed consent to participate in the study, which was approved by the Ethics Committee of the Hospital.

HLA genotyping

HLA-DRB1* genotyping was performed using the polymerase chain reaction-sequence-specific oligonucleotide (PCR-SSO) protocol recommended by the XII International Histocompatibility Workshop. Briefly, DNA was obtained from peripheral leucocytes by classic phenol/chloroform extraction. The amplification of exon 2 of the HLA-DRB1 locus was performed by PCR using aliquots of DNA (0.5 µg) in the presence of a forward primer spanning intron-4 (CCGGATCCTTCGTGTCCCCACAGCACG) and a reverse primer spanning 88-94 bp (TC GCCGCTGCACTGTGAA). Thirty cycles were carried out with *Taq* DNA polymerase in a Perkin-Elmer Cetus DNA thermal cycler, as follows: 1 min at 95ºC (denaturing), 1 min at 55ºC (annealing) and 1 min at 72ºC (elongation). The resulting 292-bp fragment was blotted onto nylon membranes and hybridized overnight at 54ºC with a set of 18 SSO probes, labeled with digoxigenin-11-ddUTP (Boehringer Mannheim). The membranes were then incubated with a Fab anti-digoxigenin IgG (Boehringer Mannheim) and detection was performed with a chemiluminescence substrate (Lumigen PPD, Boehringer Mannheim). The dots were visualized after a 10- to 20-min exposure to Kodak XAR films at room temperature.

Statistical analysis

The distribution of HLA-DRB1* alleles was compared between IDDM patients and controls by the chi-square test with Yates correction. Odds ratios were calculated according to the formula of Woolf and, by convention, expressed as relative risks (RR). When one element was 0, the Haldane formula was used to deduce the RR. The level of significance was set at 0.05. The P value was corrected by the Bonferoni inequality method, by multiplying P by the number of alleles compared.

Results

The HLA-DRB1* phenotype and allele frequencies among IDDM patients and normal individuals are shown in Table 1 and Table 2, respectively. HLA-DRB1*03 frequency (Table 1) was significantly higher in the IDDM than in the control group (48.8% in IDDM, 18.2% in controls, RR = 4.27, $P<0.005$). The HLA-DRB1*04 frequency was also significantly higher in the IDDM group (43.9% *vs* 15.1% in controls, RR =

4.37, P<0.008). HLA-DRB1*03/*04 heterozygosity conferred a strong IDDM risk (14.6% in patients *vs* 3.0% in controls, RR = 5.44).

Homozygosity for DRB1*03 and DRB1*04 did not confer a higher risk than having a single dose of these alleles (Table 2).

In contrast, the HLA-DRB1*11 frequency was decreased in IDDM patients (7.3%) compared with the control group (26.3%), but the difference was not significant when the P value was corrected by the number of DRB1 alleles tested (P<0.02 before correction). The frequencies of the other HLA-DRB1* alleles were similar in both groups.

Discussion

Susceptibility and resistance to IDDM are controlled, in part, by HLA class II genes (3). In addition to the role of the encoded molecules (particularly HLA-DQ) in the immunopathogenesis of IDDM, these gene products also serve as genetic markers that are widely used to identify individuals with a risk of developing IDDM (12,13).

Little is known about class II HLA genes and IDDM in Brazilians. In the present study, we used HLA genotyping to perform DRB1* analysis in IDDM patients and normal individuals from Southeastern Brazil. There was an increased frequency of HLA-DRB1*03 and *04 among IDDM patients compared to the controls. These results are in agreement with data obtained for other Caucasian, African and Oriental groups (14-16). The strong IDDM risk conferred by DRB1*03/*04 heterozygosity in the Brazilian IDDM group is of the same order of magnitude as that observed in Caucasian patients (14,16).

We found a tendency to a lower frequency of HLA-DRB1*11 in IDDM patients than in healthy individuals, although the difference was not significant after P correction. Other studies have previously reported the association betwen HLA-DRB1*11 and protection from IDDM in non-Caucasian

Table 1 - Distribution of HLA-DRB1 phenotype frequencies in IDDM patients and normal individuals (controls).

The frequencies are represented as a percentage of patients with each allele. P values were obtained by the chi-square test and corrected for the number of different DRB1 alleles tested (P_c, multiplication of P by 13). RR, Relative risk; NS, nonsignificant. *P<0.03 before correction; **P<0.02 before correction.

DRB1*	IDDM (N = 41)		Controls (N = 99)		RR	P_c
	N	%	N	%		
01	8	19.5	24	24.2	0.75	NS
15	5	12.2	14	14.1	0.85	NS
16	0	0.0	3	3.0	0.33	NS
03	20	48.8	18	18.2	4.27	<0.005
04	18	43.9	15	15.1	4.37	<0.008
11	3	7.3	26	26.3	0.22	* *
12	0	0.0	4	4.0	0.25	NS
13	5	12.2	14	14.1	0.85	NS
14	0	0.0	8	8.1	0.13	NS
07	5	12.2	12	12.1	1.00	NS
08	1	2.4	6	6.1	0.39	NS
09	0	0.0	3	3.0	0.33	NS
10	0	0.0	7	7.1	0.15	NS
03/04	6	14.6	3	3.0	5.44	*

Table 2 - Distribution of HLA-DRB1 allele frequencies in IDDM patients and normal individuals (controls).

The frequencies are represented as a percentage of each allele in relation to the total number of alleles (2n) (when a single DRB1 allele was found it was considered homozygous and was counted twice). P values were obtained by the chi-square test and corrected for the number of different DRB1 alleles tested (P_c, multiplication of P by 13). RR, Relative risk; NS, nonsignificant. *P<0.006 before correction.

DRB1*	IDDM (2n = 82)		Controls (2n = 198)		RR	P_c
	2n	%	2n	%		
01	8	9.7	30	15.1	0.62	NS
15	5	6.1	19	9.6	0.61	NS
16	0	0.0	5	2.5	0.21	NS
03	27	32.9	21	10.6	4.13	<0.0002
04	23	28.0	15	7.6	4.76	<0.0002
11	4	4.9	37	18.7	0.22	*
12	0	0.0	4	2.0	0.26	NS
13	5	0.061	18	9.1	0.65	NS
14	0	0.0	9	4.5	0.12	NS
07	7	8.5	15	7.9	1.14	NS
08	1	1.2	6	3.0	0.38	NS
09	0	0.0	4	2.0	0.26	NS
10	0	0.0	9	4.5	0.12	NS

populations such as Blacks and North-Africans (17,18). In Caucasians, protection is usually conferred by the DRB1*15-DQA1*0102-DQB1*0602 haplotype (3). However, DRB1*15 frequency was not decreased in our IDDM patients compared to controls.

The HLA-DRB1* genes found in the above Brazilian IDDM patients need to be re-evaluated in conjunction with an analysis of HLA-DQA1*, DQB1* haplotypes since the latter represent the primary HLA genes involved in susceptibility to the disease. Since the combination of DQA1 and DQB1 genes can vary among populations, detailed haplotype studies of different ethnic groups are required in order to better define the nature of the HLA-associated susceptibility and resistance to IDDM.

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