

HLA CLASS II POLYMORPHISM IN PATIENTS WITH TYPE 1 DIABETES MELLITUS FROM A BRAZILIAN RACIALLY ADMIXTURED POPULATION

Background: Several studies have demonstrated a fundamental role for the histocompatibility antigens (ie, human leukocyte antigens or HLA) in the susceptibility of, or protection to, type 1 diabetes mellitus (T1DM). However, this has not been adequately studied in racially admixed populations.

Objectives: To assess the frequency of HLA class II (DQA1, DQB1 and DRB1) associated to susceptibility or protection to T1DM in a Brazilian racially admixed with diabetes.

Methods: Cross-sectional study. The HLA genotyping was performed by a polymerase chain reaction hybridization assay. The racial groups were categorized by self-report and phenotype. The results are expressed as means and standard deviations of the mean, proportions and frequencies. The χ^2 and Fisher exact tests were used for the inferential statistics.

Results: The study population comprised 55 children and adolescents with T1DM. The phenotypic racial group classification demonstrated that, 60% were Mulattoes, 25.5% Whites, 12.7% Blacks and 1.8% from Indian ancestry. The T1DM's susceptibility was associated with an increased frequency of the HLA of risk (-DRB1*0401, -DRB1*0402, DQA1*03, -DQA1*05, -DQB1*02 e -DQB1*0302); and a small frequency of protective alleles (-DRB1*0404, -DRB1*0407, -DQA1*0201, -DQB1*0602, -DQB*0603 e -DQB1*0604) in all subjects. We found a greater frequency of the HLA-DRB1*0302 among Whites when compared to Blacks.

Conclusions: This study demonstrates that the frequency and distribution of the susceptibility and protective HLA alleles were similar to studies performed in the Brazilian Southeast and in North Americans and European Caucasians, suggesting that the genetic basis of T1DM has a common origin being little modified by racial characteristics. (*Ethn Dis*. 2009; 420-424)

Key Words: Type 1 Diabetes Mellitus, Major Histocompatibility Complex, Ethnicity

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INTRODUCTION

The diversity of the HLA-DRB1, -DQA1 and -DQB1 haplotypes in ethnically distinct populations is well-known, which can explain, at least partially, the differences in the T1DM (type 1 diabetes mellitus) incidence.¹ Most of the studies about HLA (human leukocyte antigen) and T1DM have involved European and North American Whites.² Some researchers have analyzed this association in other populations, (eg, South African,³ Jamaican,⁴ Cameroonian,⁵ or Japanese⁶). However, none of these has reported the nature of HLA and T1DM association in racially admixed populations.

Studies performed in Brazilians from a predominantly White, European ancestry have shown that the frequency and the distribution of the HLA class II alleles associated to T1DM is similar to other Western White populations.⁷⁻¹⁴ Nevertheless, none of these studies was conducted in northeastern Brazil, known for its predominantly racially mixed population. Due to this gap in knowledge, we designed a cross-sectional study to examine the frequency and distribution of HLA class II alleles in a sample of patients racially mixed in the city of Salvador, Bahia State, Brazil.

METHODS

This cross-sectional, quantitative study examined a sample of 55 young people who were followed in the

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Pediatric Endocrinology Clinic from the Faculty of Medicine of the Federal University of Bahia, in Salvador, Bahia, Brazil. Study participants were individuals, aged ≥ 1 years to ≤ 21 years, with the diagnosis of T1DM, defined according to the criteria of the American Diabetes Association.¹⁵ Signed informed consent forms, by parents or the individual if aged > 18 years, were collected. Patients (or their parents) who refused to sign the consent form or patients with neonatal, secondary diabetes or type 2 diabetes were not included in the study. The written approval by the Brazilian National Research Center and by the Ethics Committee of the participating hospitals was secured before the beginning of the project.

Racial group classification was based on two criteria. First, participants were classified through self-report as White, Black, Mulatto, Asian, Indian, or without self-report, participants were classified according to the methodology used by the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística- IBGE).¹⁶ Second, using methods by Krieger et al,¹⁷ we used phenotypic analysis to classify the population into five groups: White,

Light Mulatto, Medium Mulatto, Dark Mulatto and Black.¹⁷⁻²⁰

We drew 10–15 mL of venous blood from each participant. Blood samples were drawn in filter paper in order to run molecular tests. The venous blood was stored at -20°C until the laboratory evaluation. After drying at room temperature, the blood samples collected in filter paper were conditioned in individual envelopes and stored in the refrigerator at 2 to 8°C (35.6 to 46.4°F). The HLA analysis used methodology of the PerkinElmer laboratory (Turku, Finland).²¹⁻²⁴

The determination of HLA alleles was based on the DNA amplification of the loci HLA-DQB1, -DQA1 and -DRB1 according to the PCR (polymerase chain reaction) parameters, followed by the hybridization assays with the use of fluorescent lanthanide chelate conjugated oligonucleotide probes specific to the implied alleles. Lanthanides are rare earth elements whose chelates in these marked oligonucleotides are not fluorescent until hybridization, when they express the property to become highly fluorescent on fluorimetric detection, a highly sensitive process. The lanthanide chelates used were Samarium (Sm), Europium (Eu) and Terbium (Tb). The hybridization assay of the HLA-DQA1 consisted of a group of 3 probes of specific sequence oligonucleotides which allow identification of the HLA-DQA*10201, 03 (*0301 e *0303) and 05 (*0501 e *0505) alleles; the HLA-DQB1 used a group of 5 probes in order to define the presence of the HLA-DQB1*02, *0301, *0302, *0602, *0603 e *0604 alleles; and the HLA DRB1 employed a group of 5 probes to show the presence of the HLA-DRB1*04 (*0401-*0407) alleles.

This study was focused on detecting only HLA class II alleles (-DQA1, -DQB1 and -DRB1) already known to be associated with T1DM, evaluating both protection (-DQB1*0602, -DQB1*0603, -DQB1*0604, -DRB1*0404, -DRB1*0407 e -DQA1*0201) and susceptibility alleles

(-DRB1*401,-DRB1*0402, -DQA1*03, -DQA*05, -DQB1*02 e -DQB1*0302).

The descriptive analysis of the results made use of average and standard deviation to depict continuous variables and proportion and simple frequencies to illustrate qualitative variables. The statistical inference from qualitative variables was performed by χ^2 test or Fisher's Exact Test. In the statistical analysis of data, a $P < .05$ was considered significant.

RESULTS

The sample of patients was composed of 55 children and adolescents with T1DM. The age group ranged from 1 to 21 years-old (average 11.5 ± 4.7), being 26 (47.3%) female and 29 (52.7%) male. The average age of T1DM onset was 7.1 ± 3.6 years. Concerning age group, 18 patients (32.7%) were diagnosed before age 5, 22 (40%) were diagnosed between age 5 and 15 years and 15 (27.3%) were diagnosed after 15 years.

The distribution of ethnic group according to self-report was 14 White (25.5%), 7 Black (12.7%), 33 Mulattoes (60%) and 1 Indian (1.8%). There were not any Asian or non specified self-reported person.

Due to the small size of the sample, the phenotypic classification of Krieger et al¹⁷ was grouped in three categories (White, Black and Mulatto) as: 16 White (29.0%), 3 Black (5.45%) and 36 Mulattoes (65.4%).

The distribution of the allelic frequency showed a low frequency of alleles largely recognized as associated with protection against T1DM (-DRB1*0404, -DRB1*0407, -DQA1*0201, -DQB1*0602 and -DQB1*0603); and a high frequency of alleles predisposing to susceptibility (-DRB1*0401, -DRB1*0402, -DQA1*03, -DQA1*05, -DQB1*02 and -DQB1*0302) (Table 1). The most frequent susceptibility alleles were the -DQA1*03 (76.4%) and the -DQB1*0302 (71.8%) alleles;

and the less frequent protection alleles were the -DRB1*0404 (0%) and the -DRB1*0407 (1.8%). The most frequent genotypes were -DRB1*0401/*0402, -DQA1*03/05, -DQA1*03/- and -DQB1*02/*0302.

The association of the HLA-DRB1, -DQA1 e -DQB1 alleles with self-reported racial classifications groups is portrayed in Table 2. The -DQB1*0302 susceptibility allele was more frequent in White than Black or Mulattoes children, the same occurring to the -DQB1*0602 protection allele, which was more frequent in Whites as well. Nonetheless, none of these frequencies differed significantly among the three groups.

The frequency rate of the HLA-DRB1, -DQA1 e -DQB1 alleles, according to the Krieger et al¹⁷ phenotypic classification is shown in Table 3. In this categorization, the comparison two-to-two groups evidenced a higher frequency of the -DQB1*0302 allele among White vs Black children, 87.5% vs 0% ($P = .031$), but not higher in Whites than Mulattoes 87.5% vs 17.2% ($P = .26$).

DISCUSSION

The Brazilian population is genetically highly diversified, owing to the admixture of three large groups: White, Black and Indian.²⁵⁻²⁶ Most of the Caucasians came from Portugal, Spain, Italy and Germany. The Africans, brought to Brazil in the colonial times, belonged mainly to Bantu-speaking groups from equatorial Africa; and the Indians originated predominantly from Tupi and Tapuia tribes.²⁷ A Mulatto population dominates the city of Salvador; its origins resulted from the miscegenation of Caucasians from a predominantly Portuguese background, with some contribution of English and Dutch in the 16th and 17th centuries, and Blacks of Bantu and Sudanese groups.¹⁷ Due to the large territorial area of Brazil and to the diverse colonial

Table 1. Distribution of HLA class II frequencies associated to susceptibility and protection to type 1 diabetes mellitus

Locus	Protection		Susceptibility	
	Allele	N (%)	Allele	N (%)
-DRB1 (n=55)	*0404	0 (0%)	*0401	31 (56.4%)
	*0407	1 (1.8%)	*0402	25 (45.5%)
-DQA1 (n=55)	*0201	3 (5.4%)	*03	42 (76.4%)
			*05	26 (47.3%)
-DQB1 (n=39)	*0602	4 (10.2%)	*02	16 (41%)
	*0603	4 (10.2%)	*0302	28 (71.8%)

history in each of its regions, each Brazilian region has a higher or lower predominance of each of these ethnic groups.

Susceptibility and protection for T1DM are controlled, partially, by HLA class II genes. Besides its role in immunopathogenesis, the products of these genes are genetic markers, which identify people under risk of developing T1DM.^{28,29} In Brazil, only eight studies examined the relationship between HLA and T1DM; all of the studies were conducted in the southeastern part of the country, known for its predominantly European descendant population and, obviously distinct from the population in the city of Salvador, where the genetic contribution of Africans is more evident.

Data from the Brazilian Institute of Geography and Statistics (IBGE), collected from a self-report survey have shown that in 2000, the population of Salvador comprised White (23%), Mulatto (54%), Black (20.4%), Indian (0.3%) and non specified (0.68%).¹⁶ The patients of this study presented a racial group distribution similar to IBGE data: White (23%), Mulatto (60.0%), Black (12.4%) and Indian (1.4%), representing a similar racial group distribution as the population in the city where the study was done. When the phenotypic evaluation was utilized, a slight increase was observed in the number of Whites (29%), a decrease, by half, of the number of Blacks (5%), and a small increase in Mulattoes (65%). These data show the

Table 2. HLA-DRB1, -DQA1 e -DQB1 allelic association in patients with type 1 diabetes mellitus according to self-reported racial group

Association	Racial Group (self-report)			P
	Whites (n=14)	Blacks (n=7)	Mulattoes (n=33)	
Protection				
-DRB1*0404	0 (0%)	0 (0%)	0 (0%)	-
-DRB1*0407	1 (7.1%)	0 (0%)	1 (3%)	.63
-DQA1*0201	0 (0%)	0 (0%)	3 (9.1%)	.70
-DQB1*0602	2 (22.2%)	0 (0%)	2 (6%)	.59
-DQB1*0603	1 (11.1%)	1 (14%)	4 (12.1%)	>.99
Susceptibility				
-DRB1*0401	7 (50%)	5 (71.4%)	18 (54.6%)	.73
-DRB1*0402	5 (37.5%)	3 (42.9%)	16 (48.5%)	.79
-DQA1*03	11 (78.1%)	5 (71.4%)	25 (75.4%)	>.99
-DQA1*05	8 (57.2%)	5 (71.4%)	12 (36.3%)	.15
-DQB1*02	3 (33.3%)	2 (50%)	11 (42.3%)	.83
-DQB1*0302	7 (77.8%)	1 (25%)	6 (23.0%)	.06

P: χ^2 and Fisher exact test.

PS: 5 Whites, 3 Blacks and 7 Mulattoes were not tested for the -DQB1 allele.

restrictions of the racial group categorization, whose strict phenotypic criteria such as that from Krieger et al¹⁷ are biased toward enhancing the number of racially admixed patients.

In the current study only HLA class II, previously recognized as associated with T1DM, were investigated. These can be divided in protection (-DQB1*0602, -DQB1*0603, -DQB1*0604, -DRB1*0404, -DRB1*0407 e -DQA1*0201) and susceptibility (DRB1*0401, -DRB1*0402, -DQA1*03, -DQA1*05, -DQB1*02 e -DQB1*0302) alleles.³⁰ This study did not evaluate either the HLA-DRB1*03 (DR3) susceptibility allele or other protection alleles such as the -DRB1 (*11, *13, *1501 [DR2]), the -DQA1*0102 and -DQB1*0501 alleles.

The HLA-DQB1*0302 and -DQA1*03 alleles, considered as the main susceptibility genes to T1DM in the Western medical literature,³¹ was found in 71.8% and 76.4% of the patients, respectively, reassuring its role in the predisposition to disease. Similar results were described by Volpini et al,⁸ in the southeast of Brazil, where the researchers found a relative risk of 3.3 to the -DQB1*0302 allele. Our study found an increase in the susceptibility to T1DM associated with the HLA-DRB1*0401 (56.4%) and -DRB1*0402 (45.5%) alleles, similar to studies developed in the Southeast of the country.⁷⁻¹¹ The HLA-DQB1*02 and -DQB1*0201 alleles were associated with susceptibility in the studies of Volpini et al⁸ and Fernandes et al¹¹ and in our study, in which the frequency of the -DQB1*02 allele was present in 41% of the patients. Although the HLA-DR3 (-DRB1*03) allele has not been investigated, it was associated with susceptibility to T1DM in Brazilians in the southeastern part of the country, as well as in other Caucasian populations in the United States and Europe.⁸⁻¹¹ The finding of susceptibility alleles to T1DM in a similar frequency to other reports from Brazil achieves our goal to demonstrate that racially admixed and

Table 3. HLA-DRB1, -DQA1 e -DQB1 association in patients with type 1 diabetes mellitus according to the racial group evaluated by phenotype (Krieger et al, 1965)

Association	Racial group (phenotype)			P
	Whites (n=16)	Blacks (n=3)	Mulattoes (n=36)	
Protection				
-DRB1*0404	0 (0%)	0 (0%)	0 (0%)	-
-DRB1*0407	1 (6.25%)	0 (0%)	1 (3.0%)	.57
-DQA1*0201	1 (6.25%)	0 (0%)	2 (6.0%)	>.99
-DQB1*0602	0 (0%)	0 (0%)	4 (15.3%)	.44
-DQB1*0603	1 (12.5%)	1 (50%)	3 (11.5%)	.27
Susceptibility				
-DRB1*0401	9 (56.3%)	3 (100%)	19 (52.8%)	.37
-DRB1*0402	6 (37.6%)	1 (33.1%)	18 (50.0%)	.73
-DQA1*03	12 (75.0%)	1 (33.3%)	29 (80.6%)	.17
-DQA1*05	8 (50.0%)	2 (66.6%)	16 (44.5%)	.74
-DQB1*02	2 (25.0%)	2 (66.6%)	12 (41.4%)	.09
-DQB1*0302	7 (87.5%)	0 (0%)	5 (17.2%)	.04

P: Fisher exact test.

PS: 8 Whites, 1 Black and 7 Mulattoes were not tested for the HLA-DQB1 allele.

Caucasian populations have the same pattern of HLA class II susceptibility to T1DM.

The HLA-DRB1*0404 and *0407 alleles, considered as protection alleles to T1DM in Brazilians from the southeast and in Caucasian populations,⁸ maintained the same property in our study, where its frequencies were, 0% and 1.8%, respectively. The HLA-DQB1*0602 allele, found in just 10.2% of our patients, was protective in the population studied by Volpini et al⁸ and Fernandes et al.¹¹ Another protection allele was the -DQB1*0603 allele, which was present in 10.2% of the patients with diabetes.

As it has been largely reported in the Western medical literature, the heterozygous genotype HLA-DRB1*03/*04

(DR3/DR4) was associated with susceptibility in the studies performed in Brazil.^{7-11,13} As the -DR3 was not investigated in our study, we can not make this comparison. In the current study the genotypes most associated with T1DM were the HLA-DQA1*03/-, -DQA1*03/05, -DQB1*02/*0302 and -DRB1*0401/*0402. Likewise, in Finland, the genotype -DQB1*02/*0302 was most strongly associated with T1DM, with a frequency of 28.8% compared to 2.9% in individuals without diabetes.^{24,32}

Concerning genetic background of Brazilian racial groups without T1DM, Moraes et al²⁷ studied Brazilians without T1DM in the Rio de Janeiro state, categorized in two groups: White and Black. Its outcomes displayed the absence of the HLA-DRB1*0401 e -DRB1*0404 alleles in Blacks and a 9.3% and 10.4% frequency rate in Whites. Contrasting with this data, 56.4% of our population with T1DM had the HLA-DRB1*0401 allele, which reinforces its presence in persons at higher risk for T1DM.

We did not demonstrate significant differences between the frequency of susceptibility alleles to T1DM in our different racial groups (as defined by the

Krieger et al classification method¹⁷), except for a higher frequency of HLA-DQB1*0302 allele in Whites than in Blacks, but not higher in Whites than in Mulattoes. However, the small number of patients in the comparison groups hampers its clinical validation. Reitnauer et al³³ did not find differences in the association between HLA antigens and T1DM between Black and American Whites. Furthermore, African studies also have not shown significant differences in the susceptibility alleles to T1DM when Black populations (eg, South Africa, Cameroon, Zimbabwe, Ethiopia) were compared with Caucasian.^{3,5} As of 2008, the only ethnic difference related to risk of T1DM was confirmed in Japanese people, whose frequency of the -DRB1*03/DQB1*0201 genotype is almost absent and the -DRB1*04/-DQB1*0302 is rare.³⁴

Our study had a few limitations. First, if we had focused the research only on all HLA alleles known to be associated with susceptibility and protection to T1DM. The (HLA-DRB1, -DQA1 and -DQB1) genotype, we would have been able to study the haplotypes associated with T1DM. Secondly, if we had conducted a case-control study, it would have permitted a comparison of the available data in the patients with healthy controls. Nevertheless, this study is the first to indicate the distribution and frequency of HLA class II alleles associated with T1DM in a racially admixed population, suggesting that the genetic basis of T1DM has a common origin being little or not affected by racial groups differences.

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*In the current study the genotypes most associated with T1DM were the HLA-DQA1*03/-, -DQA1*03/05, -DQB1*02/*0302 and -DRB1*0401/*0402.*

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