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Frequency distribution of XbaIG > T and HaeIIIT > C *GLUT1* polymorphisms among different Brazilian ethnic groups

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Abstract GLUT is the major glucose transporter in mammalian cells. Single nucleotide polymorphisms (SNP) at *GLUT1* promoter and regulatory regions have been associated to the risk of developing nephropathy in different type 1 and type 2 diabetic populations. It has been demonstrated that differences in allelic and genotypic frequencies of *GLUT1* gene (SLC2A1) polymorphisms occur among different populations. Therefore, ethnic differences in distribution of *GLUT1* gene polymorphisms may be an important factor in determining gene-disease association. In this study, we investigated the XbaIG > T and HaeIIIT > C polymorphisms in six different Brazilian populations: 102 individuals from Salvador population (Northern Brazil), 56 European descendants from Joinville (South Brazil), 85 Indians from Tiryió tribe (North Brazil) and 127 samples from Southern Brazil: 44 from European descendants, 42 from African descendants and 41 from Japanese descendants. Genotype frequencies from both sites did not differ significantly from those expected under the Hardy–Weinberg equilibrium. We verified that the allele frequencies of both polymorphisms were heterogeneous in these six Brazilian ethnic groups.

Keywords GLUT1 · Polymorphisms · Brazilian populations

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Introduction

Mammalian cells usually require blood glucose as their major source of energy and this molecule is transported into the cell by the glucose transporters (GLUT). These transporters constitute a family of 13 members which facilitate basal glucose transport into cells. GLUT1, a member of the class I family of glucose transporters together with GLUT2, GLUT3, GLUT4 and GLUT14, is a uniport carrier that passively facilitates glucose transport across membranes. GLUT1 is widely expressed as it is the main glucose transporter in the brain, placenta and erythrocytes [1]. The *GLUT1* gene is located on chromosome 1 at 1p31–35.2 and single nucleotide polymorphisms (SNP) in its promoter (−2841 A > T) and regulatory (XbaIG > T, HaeII Enhancer-2 SNP1) regions have been implicated in the risk of developing nephropathy in different type 1 and type 2 diabetic populations [2–7]. In addition, it has been previously demonstrated that differences in allelic and genotypic frequencies of *GLUT1* gene polymorphisms occur among different populations [2–7], for example, the XbaIG > T polymorphism shows a frequency of the G allele of 55.0% in African–American population and of 79.0% in Chinese population [5, 8]. This ethnic diversity

may be an important factor in determining gene-disease association [1].

Brazil is a South American nation characterized by the admixture of several ethnic groups such as the Portuguese, Africans, indigenous tribes and a variety of other European immigrants. It has become an ethnic, genetic and cultural diverse nation [9, 10]. However, the contribution of these different ethnic groups varies among the different regions of Brazil [11].

Because variations in *GLUT1* polymorphisms have been associated to disease susceptibility among various ethnic groups and because there is no information about *GLUT1* polymorphisms in Brazilians, we decided to investigate the distribution of the XbaIG > T and HaeIIIT > C polymorphisms at *GLUT1* gene in distinct Brazilian populations with different ethnic backgrounds.

Materials and methods

Populations studied

We examined 370 genomic DNA samples from distinct populations of Brazil that were collected between 1997 and 2002 as part of previous studies: 102 samples randomly selected from the general population of Salvador, Bahia [12], ~80% of which have mixed and Portuguese ancestry [13], representing the Brazilian Northern region; 56 randomly selected European descendants from Joinville, Santa Catarina blood center (South Brazil), which have a high European contribution in the gene pool region, especially Germans [14]; 85 samples also randomly selected from the Indians from Tiryió tribe who live isolated in North Brazil along the border with Suriname [15] and 127 samples from Southern Brazil (São Paulo state): 44 samples collected from European descendants, 42 from African descendants and 41 from Japanese descendants. In the last group, the criteria established for their selection was based on the pure family ascendancy in the two preceding generations and absence of kinship among them [16].

DNA extraction and single nucleotide polymorphism detection

Genomic DNA was extracted from peripheral blood mononuclear cells using a proteinase K treatment followed by a phenol–chloroform method [17]. Immediately after the extraction, the DNA samples were storage at −20°C in a DNA exclusive freezer. The analysis of the XbaIG > T and HaeIIIT > C polymorphisms was performed through PCR amplification followed by restriction fragment length polymorphism (RFLP). The XbaIG > T polymorphism was performed using the primers 5′-TGTGCAACCC ATGAGCTAA-3′(F) and 5′-CCTGGTCTCATCTGGATT CT-3′(R), and after 35 cycles of amplification consisting of denaturation at 95°C for 45″, annealing at 55°C for 45″ and extension at 72°C for 90″, the 1.1 kb PCR products were digested with *Xba*I restriction enzyme in 37°C overnight. The XbaIG > T polymorphism consists of a G-to-T substitution which abolishes a recognition site for the *Xba*I restriction enzyme. The resulting digested fragments were separated by gel electrophoresis on a 1.2% agarose gel and scored under ultraviolet light [18]. The genotyping success rate was 92%. The HaeIIIT > C polymorphism was performed using the primers 5′-CTCCCAGACACGCCTAT AACAGT-3′ (F) e 5′-GGCTGGTGTCCATAAGCCAA CG-3′ (R), and the termocycling parameters were: denaturation at 95°C for 45″, annealing at 66°C for 45″ and extension at 72°C for 60″. After 30 cycles of amplification, the 173 bp PCR products were digested with *Hae*III restriction enzyme in 37°C overnight. The HaeIIIT > C polymorphism consists of a C-to-T substitution that creates a recognition site for the *Hae*III restriction enzyme. The fragments were electrophoresed on a 2.0% agarose gel and visualized with ethidium bromide [18]. The genotyping success rate was, ~98%.

Statistical methods

The allelic frequencies were estimated by direct allele counting. The conformity with Hardy–Weinberg equilibrium and existence of linkage disequilibrium were tested using the Genepop v.3.4 [19]. The heterogeneity between population samples was evaluated by Fisher's exact test or by χ^2 test. A P value of <0.05 was considered statistically significant.

Results

The distribution of *GLUT1* polymorphisms frequencies in the six populations studied is shown in Tables 1 and 2. Genotypic and allelic frequencies from the analyzed sites did not differ significantly from those expected under Hardy–Weinberg equilibrium, except for the XbaIG > T polymorphism on the Amerindians from Tiryió tribe ($P = 0.0459$). We observed a strong linkage disequilibrium between the HaeIIIT > C and XbaIG > T polymorphisms in the Salvador population ($P = 0.00016$) and European descendants from South Brazil ($P = 0.0152$). The allele frequencies of the XbaIG > T ($P < 0.001$) and HaeIIIT > C ($P < 0.001$) polymorphisms differed among the regional Brazilian populations: the Indians from Tiryió tribe presented the lowest XbaIG allele frequency (52.4%) and the Japanese descendants had the highest XbaIG allele frequency (79.3%) (Table 1). A similarly heterogeneous

Table 1 Genotypic and allelic frequencies of XbaIG > T polymorphism in Brazilian populations

XbaIG > T	Genotypes			Alleles	
	G/G (%)	G/T (%)	T/T (%)	G (%)	T (%)
African descendants[a]	14(33.3)	22(52.4)	6(14.3)	50(59.5)	34(40.5)
Salvador population[b]	29(28.0)	57(56.0)	16(16.0)	115(56.0)	89(44.0)
European descendants					
South region[c]	25(44.6)	24(42.9)	7(12.5)	74(66.1)	38(33.9)
Southeast region[d]	27(61.4)	13(29.5)	4(9.1)	67(76.1)	21(23.9)
Japanese descendants[e]	25(61.0)	15(36.6)	1(2.4)	65(79.3)	17(20.7)
Tiryió Indians[f]	28(32.9)	33(38.8)	24(28.3)	89(52.4)	81(47.6)

[a, d, e] Descendants from Ribeirão Preto city, São Paulo state (Southeast of Brazil)

[b] Salvador population from Salvador city, Bahia state (Northeast of Brazil)

[c] European descendants from Joinville city, Santa Catarina state (South of Brazil)

[f] Indians from Tiryió tribe, Roraima state (North of Brazil)

Table 2 Genotypic and allelic frequencies of HaeIIIT > C polymorphism in Brazilian populations

HaeIIIT > C	Genotypes			Alleles	
	T/T (%)	T/C (%)	C/C (%)	T (%)	C (%)
African descendants[a]	0(0.0)	13(30.9)	29(69.1)	13(15.5)	71(84.5)
Salvador population[b]	1(1.0)	36(35.0)	65(64.0)	38(18.6)	166(81.4)
European descendants					
South region[c]	3(5.4)	23(41.1)	30(53.5)	29(25.9)	83(74.1)
Southeast region[d]	3(6.8)	12(27.3)	29(65.9)	18(20.5)	70(79.5)
Japanese descendants[e]	0(0.0)	20(48.8)	21(51.2)	20(24.4)	62(75.6)
Tiryió Indians[f]	17(20.0)	48(56.5)	20(23.5)	82(48.2)	88(51.8)

[a, d, e] Descendants from Ribeirão Preto city, São Paulo state (Southeast of Brazil)

[b] Salvador population from Salvador city, Bahia state (Northeast of Brazil)

[c] European descendants from Joinville city, Santa Catarina state (South of Brazil)

[f] Indians from Tiryió tribe, Roraima state (North of Brazil)

pattern was observed in the HaeIIIT > C polymorphism frequencies. The lowest frequency of the HaeIIIT allele was found in the European descendants from Southern Brazil (15.5%) and in the highest frequency in the Tiryió tribe (48.2%) (Table 2).

Table 3 *P* value of the allelic frequencies of XbaIG > T polymorphism in the studied Brazilian populations

Alleles	*P* value*
Indians × European descendants (South region)	0.022
Indians × European descendants (Southeast region)	<0.0001
Indians × Japanese descendants	<0.0001
European descendants (South region) × Japanese descendants	0.044
European descendants (Southeast region) × African descendants	0.020
European descendants (Southeast region) × Salvador population	0.001
Japanese descendants × African descendants	0.006
Japanese descendants × Salvador population	<0.0001

* Allelic frequencies with statistical significance (*P* value < 0.05)

The allele frequencies of the XbaIG > T polymorphism differed significantly among populations as shown in Table 3. In relation to the HaeIIIT > C polymorphism, we only observed a significant difference in the allelic frequency distribution between Tiryió tribe and the other five groups studied ($P < 0.0001$).

Discussion

Many studies that estimate the ethnic admixture in Brazilians have shown that the contribution of each ethnic group in the gene pool formation varies according to the Brazilian regions [11]. Brazil was colonized by representatives of different ethnic groups including Europeans, Africans and the autochthonous Amerindians. The South is predominantly composed of European descendents. The Germany influence, for example, was important in Joinville, a city located in Santa Catarina State. The North region shows a high contribution from both the Amerindians and Europeans, and a lower contribution of Africans. In Northern Brazil, the most important contribution to the gene pool region is from Europeans and Africans, and there is a smaller influence of Amerindians, as shown in the city of Salvador (capital of the state of Bahia) [20]. In this study, we show a different distribution of the XbaIG > T and HaeIIIT > C *GLUT1* polymorphisms in different Brazilian populations.

Differences in the distribution of SNP can be observed in populations with different ethnic backgrounds [21, 22]. Our previous studies have also shown that allelic frequency of polymorphisms in the Interleukins 6 [22] and 10 (data not shown) varies among Brazilian regions.

Previous results have shown that the XbaIG allele frequency is highly heterogeneous among different populations (Table 4). The high frequency of XbaIG allele in

Table 4 Genotypic and allelic frequencies of XbaIG > T polymorphism in European, Oriental and African–American populations

XbaIG > T	Genotypes (%)			Alleles (%)	
	G/G	G/T	T/T	G	T
European					
England[a]	20.2	57.7	22.1	49.0	51.0
Poland[b]	43.0	42.0	15.0	64.4	35.6
Italians[d]	46.5	45.2	8.3	69.0	31.0
Chinese[c]	62.0	33.0	5.0	79.0	21.0
Orientals[d]	68.9	27.0	4.1	82.0	18.0
African–American[d]	31.0	48.3	20.7	55.0	45.0

[a] Hodgkinson et al. [2]

[b] Grzeszczak et al. [4]

[c] Liu et al. [5]

[d] Pontiroli et al. [8]

Japanese descendants from Brazil is consistent with those observed previously in Chinese and Japanese populations (Table 4). This allele showed a similar frequency in the African descendants from Southern Brazil and the Salvador population (Table 1). These data are consistent with those observed previously in African–American populations (Table 4).

The XbaIT allele frequency in the European descendants from South Brazil is higher than that observed in the European descendants from Southern Brazil (Table 1). This result could be due to the existing variation in the allele frequency of the XbaIG > T polymorphism in the different European populations (Table 4) and to the different European colonization which occurred in these two regions: the South had predominantly German colonizers and Southern Brazil was predominantly colonized by Italians.

As regards the difference in the allelic frequency of the XbaIG > T polymorphism observed between Indians from Tiryió tribe and Japanese descendants (Table 1), this can be explained by the practice of endogamy in the Tiryió tribe.

In relation to the HaeIIIT > C polymorphism, we also detected great heterogeneity among Brazilian samples and a significant difference in the allelic frequency distribution between Indians from Tiryió tribe and the other five groups studied. This last observation may also be due to the practice of endogamy in the Tiryió tribe resulting in similar frequencies of both alleles (T and C) analyzed.

Few studies have investigated the HaeIIIT > C gene polymorphism and all of them were carried out in European and Japanese populations. Our genotype frequencies of the European and Japanese descendants from the Southern Brazil were similar to those observed in previous studies [18, 24].

GLUT1 gene polymorphisms have been associated with the development of nephropathy in different type 1 and type 2 diabetic populations and differences in allelic and genotypic frequencies of *GLUT1* gene polymorphisms occurring among different populations [2–8]. In this study, we confirmed that the polymorphic *GLUT1* gene alleles are influenced by ethnicity among the different groups studied. Our results are consistent with those obtained in other populations with the same ethnic background. However, it still remains to be proven that differences in the XbaIG > T and HaeIIIT > C *GLUT1* allele distributions among ethnic groups contribute to population variance in disease susceptibility.

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