

## HLA-A, B and DRB1 allele and haplotype frequencies in volunteer bone marrow donors from the north of Parana State

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**Background:** Knowledge of allele and haplotype frequencies of the human leukocyte antigen (HLA) system is important in the search for unrelated bone marrow donors. The Brazilian population is very heterogeneous and the HLA system is highly informative of populations because of the high level of polymorphisms.

**Aim:** The aim of this study was to characterize the immunogenetic profile of ethnic groups (Caucasians, Afro-Brazilians and Asians) in the north of Parana State.

**Methods:** A study was carried out of 3978 voluntary bone marrow donors registered in the Brazilian National Bone Marrow Donor Registry and typed for the HLA-A, B and DRB1 (low resolution) loci. The alleles were characterized by the polymerase chain reaction sequence-specific oligonucleotides method using the LabType SSO kit (One Lambda, CA, USA). The ARLEQUIN v.3.11 computer program was used to calculate allele and haplotype frequencies.

**Results:** The most common alleles found in Caucasians were HLA-A\*02, 24, 01; HLA-B\*35, 44, 51; DRB1\*11, 13, 07; for Afro-Brazilians they were HLA-A\*02, 03, 30; HLA-B\*35, 15, 44; DRB1\*13, 11, 03; and for Asians they were: HLA-A\*24, 02, 26; HLA-B\*40, 51, 52; DRB1\*04, 15, 09. The most common haplotype combinations were: HLA-A\*01, B\*08, DRB1\*03 and HLA-A\*29, B\*44, DRB1\*07 for Caucasians; HLA-A\*29, B\*44, DRB1\*07 and HLA-A\*01, B\*08 and DRB1\*03 for Afro-Brazilians; and HLA-A\*24, B\*52, DRB1\*15 and HLA-A\*24, B\*40 and DRB1\*09 for Asians.

**Conclusion:** There is a need to target and expand bone marrow donor campaigns in the north of Parana State. The data of this study may be used as a reference by the Instituto Nacional de Câncer/Brazilian National Bone Marrow Donor Registry to evaluate the immunogenetic profile of populations in specific regions and in the selection of bone marrow donors

**Keywords:** HLA antigens; Transplantation; Genetic polymorphism; Gene frequency

### Introduction

Human Leukocyte Antigens (HLA) are encoded by genes located on the short arm of chromosome 6 in the Major Histocompatibility Complex (MHC) region. The two types of polymorphic MHC genes, class I and II, encode proteins responsible for presenting processed peptides to T cells. HLA class I molecules (HLA A, B and C) are recognized by CD8<sup>+</sup> T cells and class II molecules (HLA - DR, DQ, DP) by CD4<sup>+</sup> T cells.<sup>(1)</sup> The HLA molecules are the main antigens that differ between individuals of the same species and are related to rejection in solid organ transplantation and graft-versus-host disease in hematopoietic stem cell transplantation.<sup>(2)</sup> The study of gene frequency of HLA alleles is important in both related and unrelated bone marrow transplantation programs and is based on HLA compatibility to prevent graft rejection and in the search for suitable donors.

In Brazil, the Instituto Nacional do Câncer (INCA) is the organ of the Ministry of Health responsible for the development and coordination of integrated measures to prevent and control cancer in Brazil and maintain strategies and programs related to the development and maintenance of REDOME (Registry of Bone Marrow Donor) and REREME (Registry of Bone Marrow Recipients) thus linking the results of HLA typing of donors in REDOME and test results stored in REREME when a compatible related donor is not found.

The genetic constitution of the Brazilian population presents peculiarities that vary according to region. In colonial Brazil, the Spanish were the first to settle the region of Parana; however today the majority of the inhabitants of the state are descendants of the Portuguese. There are also ancestors of immigrants from other countries such as

Conflict-of-interest disclosure:  
 The authors declare no competing  
 financial interest

Submitted: 11/12/2010  
 Accepted: 11/29/2011

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DOI: 10.5581/1516-8484.20120010

Italy, Germany, Poland, Ukraine, Japan and the Middle East.<sup>(3)</sup> Data from the Brazilian National Household Sample Survey (PNAD/2000) state that the population of Londrina is comprised of Caucasians (74.2%), Mulattos (18.3%), Afro-Brazilians (2.8%), Asians (3.6%) and Amerindians (0.3%). According to the Brazilian Institute of Geography and Statistics (IBGE) 2008 Census, the main immigrant groups in Londrina and the north of Parana State are Italian, Spanish, Portuguese, German and Japanese.<sup>(4)</sup> The Italian consulate in Londrina estimates that more than one third of the inhabitants of the north of Parana State are descendants of Italians and thus is the largest ethnic group in the region.

Because of the importance of knowing the genetic variability of the HLA system and that the probability of finding an HLA-matched donor depends on the number of people registered in REDOME, on the haplotype diversity and on the allele distribution of donors, the aim of this study was to estimate the HLA-A, B and DRB1 allele and haplotype frequencies in three ethnic groups and to characterize the immunogenetic profile of donors of the Regional Blood Bank in Londrina, Hospital Universitário/Universidade Estadual de Londrina.

The participants of this study were volunteer bone marrow donors from the region of Londrina and the north of Parana State registered in REDOME.

## Methods

### Population

A cross-sectional study was performed of a group of 3978 voluntary bone marrow donors at the Regional Blood Bank in Londrina, Hospital Universitário/Universidade Estadual de Londrina, registered in REDOME in the period from April 2008 to February 2010. The population was divided into three ethnic groups: Caucasians (n = 3295), Afro-Brazilians (n = 538) and Asians (n = 145). Data relating to the ethnicity and age were reported by donors and all participants signed consent forms. This research project was approved by the Research Ethics Committee of the Universidade Estadual de Londrina.

Blood samples (4 mL) were collected in sterile tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and sent to the Histocompatibility Laboratory of the Hospital Universitário de Londrina to type the HLA-A, B and DRB1 alleles at low and medium resolution.

### DNA extraction

Aliquots of 100 µL of peripheral blood were used for the extraction of genomic DNA, using the mini-spin column method with the NeoScience NeoIsoColumn® (One Lambda, San Diego, CA, USA) and Biopur Kits (Biometrix, Brazil, Parana, Curitiba) following the instructions provided by the manufacturers.

### Class I and II HLA typing

HLA genotyping was performed by the polymerase chain reaction-sequence of specific oligonucleotides (PCR-SSO) method using the LabType SSO kit (One Lambda, CA, USA) and Luminex Labscan 100 flow cytometer with software (One Lambda, San Diego, CA, USA). The DNA samples were amplified utilizing a mixture of Taq DNA polymerase (5 U/mL; Invitrogen Carlsbad, CA, USA), D-mix and primers for the A, B and DRB1 loci using the volumes recommended for the amplification of the specific loci. The PCR plate was transferred to a thermocycler (Perkin-Elmer 9600 - Waltham, Massachusetts, USA) for the amplification of exons 2 and 3 of the A and B loci and exon 2 of the DRB1 locus as recommended by the manufacturer.

The amplified DNA fragments were separated by electrophoresis in a Micro SSP Gel System (One Lambda® - San Diego, CA, USA) in 2.0% agarose gel at 150 volts for 10 minutes.

The amplified exons were hybridized using probe-conjugated beads, which have the same genetic information as the amplified exon, allowing HLA genotyping. The amplicons were used for hybridization with different oligonucleotide-conjugated beads which bind to specific complementary sequences. This reaction is revealed by the binding of biotinylated probes that react with streptavidin-phycoerythrin (SAPE), which in turn binds to fluorescently-labeled beads of different colors. Data were analyzed employing the HLA Fusion software - Labtype (One Lambda® - San Diego, CA, USA).

### Statistical analysis

The ARLEQUIN computer program version 3.11 was used to calculate the allele and haplotype frequencies as well as to assess whether the population was in Hardy-Weinberg equilibrium after the data had been organized using the Convert program. The ARLEQUIN software algorithm method is considered the gold standard to estimate the allele and haplotype frequencies in populations.

## Results

A total of 60.9% of the participants were women, the mean age was 29 years old, 43.8% had completed high school education and 42.4% had been to university. Of the ethnic groups, Caucasians predominated (82.9%), followed by Afro-Brazilians (13.5%) and Asians (3.6%).

The commonest alleles within each locus were as follows:

Locus A (Table 1)

A\*02 (27%), A\*24 (10.8%), A\*03 (10.3%), A\*01 (10.2%), A\*11 (6.8%) and A\*68 (5.5%)

Locus B (Table 2)  
 B\*35 (13.9%), B\*44 (11.1%), B\*51 (9.0%), B\*15 (8.0%)  
 and B\*07 (6.8%)

Locus DRB1 (Table 3)  
 DRB1\*11 (14.7%), DRB1\*13 (13.6%), DRB1\*07 (13.0%),  
 DRB1\*04 (12.6%), DRB1\*01 (10.4%), DRB1\*03 (9.9%) and  
 DRB1\*15 (8.1%)

The most frequent haplotypes (Table 4)  
 A\*01B\*08DRB1\*03 (2.7%), A\*29B\*44DRB1\*07  
 (1.6%) and A\*02B\*35DRB1\*11 (1.3%)

*Afro-Brazilians*

Locus A (Table 1)  
 A\*02 (20.8%), A\*03 (10.3%), A\*30 (8.7%), A\*68 (8.3%),  
 A\*24 (7.5%) and A\*23 (7.1%)

Locus B (Table 2)  
 B\*35 (11.7%), B\*15 (10.7%), B\*44 (9.4%), B\*07 (7.0%)  
 and B\*51 (6.18%)

Locus DRB1 (Table 3)  
 DRB1\*13 (14.4%), DRB1\*11 (13.8%), DRB1\*03 (11.8%),  
 DRB1\*15 (11.4%), DRB1\*07 (11.2%), DRB1\*04 (9.7%) and  
 DRB1\*01 (9.1%)

The most frequent haplotypes (Table 4)  
 A\*29B\*44DRB1\*07 (1.6%) and A\*01B\*08DRB1\*03  
 (1.5%)

*Asians*

Locus A (Table 1)  
 A\*24 (31.7%), A\*02 (25.5%), A\*26 (10.7%), A\*11 (10%)  
 and A\*31 (9.3%)

Locus B (Table 2)  
 B\*40 (17.6%), B\*51 (12.4%), B\*52 (10.7%), B\*35 (7.6%),  
 B\*15 (7.2%), B\*44 (6.5%) and B\*46 (5.2%)

Locus DRB1 (Table 3)  
 DRB1\*04 (20.3%), DRB1\*15 (18.3%), DRB1\*09 (12.7%),  
 DRB1\*08 (8.7%), DRB1\*13 (8.3%) and DRB1\*01 (8.0%)

The most frequent haplotypes (Table 4)  
 A\*24B\*52DRB1\*15 (8.2%), A\*24B\*40DRB1\*09  
 (3.7%) and A\*02B\*46DRB1\*08 (3.1%)

Frequencies of more than 0.001 were considered  
 significant.

There was greater agreement in the distribution of  
 allele frequencies between the Caucasoid and Afro-Brazilian  
 populations. Significant differences were observed between  
 these groups and the HLA-B\*54 (5.2%) and B\*46 (5.2%)  
 alleles were found only in Asians.

All ethnic groups analyzed were in Hardy-Weinberg  
 equilibrium (p-value > 0.05) as shown in Table 5.

Table 2 - Frequency of alleles at the HLA-B locus

Locus B	Caucasian n = 3295	Afro-Brazilian n = 538	Asian n = 145
07	0.0683	0.0697	0.0517
08	0.0558	0.0427	0.0034
14	0.0564	0.0548	*
15	0.0798	0.1068	0.0724
18	0.0575	0.0520	*
35	0.1388	0.1171	0.0758
39	0.0318	0.0316	0.0586
40	0.0418	0.0343	0.1758
42	0.0059	0.0362	*
44	0.1111	0.0938	0.0655
46	*	*	0.0517
49	0.0322	0.0306	0.0034
51	0.0898	0.0613	0.1241
52	0.0177	0.0195	0.1068
53	0.0147	0.0418	0.0103
54	*	*	0.0517

\*frequency < 0.001

Table 1 - Frequency of alleles at the HLA-A locus

Locus A	Caucasian n = 3295	Afro-Brazilian n = 538	Asian n = 145
01	0.1022	0.0697	0.0137
02	0.2703	0.2081	0.2551
03	0.1033	0.1031	0.0137
11	0.0684	0.0446	0.1000
23	0.0273	0.0715	*
24	0.1081	0.0752	0.3172
26	0.0404	0.0269	0.1068
29	0.0459	0.0408	*
30	0.0392	0.0873	*
31	0.0374	0.0511	0.0931
32	0.0409	0.0250	*
33	0.0273	0.0344	0.0517
68	0.0548	0.0827	*

\*frequency < 0.001

Table 3 - Frequency of alleles at the HLA-DRB1 locus

DRB1 Locus	Caucasian n = 3295	Afro-Brazilian n = 538	Asian n = 145
01	0.1037	0.0910	0.0793
03	0.0986	0.1180	0.0103
04	0.1261	0.0975	0.2034
07	0.1297	0.1124	0.0275
08	0.0439	0.0659	0.1172
09	0.0130	0.0185	0.1275
10	0.0237	0.0157	*
11	0.1474	0.1384	0.0482
12	*	*	0.0379
13	0.1357	0.1440	0.0827
14	0.0463	0.0381	0.0586
15	0.0807	0.1143	0.1827

\*frequency < 0.001

Table 4 - Frequency of the most frequent haplotypes

Haplotype	Caucasian n = 3295	Afro-Brazilian n = 538	Asian n = 145
A*01B*08DRB1*03	0.0270	0.0146	0.0034
A*02B*07DRB1*15	0.0097	0.0072	*
A*02B*15DRB1*04	0.0041	0.0087	0.0068
A*02B*35DRB1*11	0.0126	*	*
A*02B*44DRB1*04	0.0110	0.0030	*
A*02B*46DRB1*08	*	*	0.0305
A*03B*07DRB1*15	0.0102	*	0.0034
A*24B*40DRB1*04	0.0018	*	0.0219
A*24B*40DRB1*09	0.0016	*	0.0373
A*24B*52DRB1*15	0.0018	*	0.0822
A*29B*44DRB1*07	0.0164	0.0157	*
A*30B*42DRB1*03	0.0015	*	*
A*33B*44DRB1*04	*	0.0027	0.0258

\*frequency &lt; 0.001

Table 5 - Hardy-Weinberg Equilibrium

Locus	Caucasian n = 3295	Afro-Brazilian n = 538	Asian n = 145
A	0.3140	0.9067	0.8934
B	0.3477	0.9391	0.5461
DRB1	0.2129	0.8959	0.1618

Statistically significant: p-value &lt; 0.05

## Discussion

The Brazilian population has a major racial admixture because of extensive immigration from other continents causing great genetic variability of the HLA system. In the current study, significant differences and similarities were found in the allele distributions between racial groups. The results of the HLA-A locus showed that the A\*02 and A\*24 alleles are common in Caucasians, Afro-Brazilians and Asians, while the A\*03 allele is common only in Caucasians and Afro-Brazilians.

In this study the distribution of these alleles is similar to the results of other studies carried out in Brazil such as of populations from Curitiba and from the north/northwest region of Parana State,<sup>(5,6)</sup> the northeast region of São Paulo State<sup>(7)</sup> and a population from the State of Pernambuco.<sup>(8)</sup> The A\*11 allele was frequently observed among Caucasians and Asians in our region and another study reported that it is widely distributed in a Chinese population.<sup>(9)</sup>

Compared to an American population,<sup>(10)</sup> the results of this study for locus A were similar for Caucasians, African descendants and Asians, but with differences in gene frequencies in groups. The results for a mixed Venezuelan population<sup>(11)</sup> and for a Germany population<sup>(12)</sup> were similar to the Caucasoid group in our region.

Comparatively, there was similarity in the genetic patterns between the Brazilian population as a whole and the northern region of Parana State in relation to the commonest alleles at the A, B and DRB1 loci in the Caucasian population. This was also similar to the results of studies from other countries such as Colombia,<sup>(13)</sup> Costa Rica (ethnically characterized as hybrid with approximately 67% of the population having European ancestors)<sup>(14)</sup> and Uruguay (A and B loci) where the majority of the population is Caucasian.<sup>(15)</sup> This similarity is possibly due to the presence of Europeans who actively participated in the colonization of these countries including Brazil as seen by available historical and demographic information.

In this study, the analysis and identification of class I alleles at the B locus showed that the B\*35, B\*44, B\*51 and B\*15 alleles were common to all three ethnical groups and that there was little variability in frequencies between Caucasians and Afro-Brazilians. There was no significant difference in the frequencies of the B\*35 allele of Caucasians (13.9%) and Afro-Brazilians (11.7%) compared to other population groups from the north/northwest region of Parana State,<sup>(5)</sup> Curitiba,<sup>(6)</sup> northeast of the State of São Paulo<sup>(7)</sup> and the State of Pernambuco.<sup>(8)</sup> In terms of genetic distance, the frequency of HLA class I alleles in the population of the state of Pernambuco<sup>(8)</sup> is closer to the population of mulattoes and Whites of the State of Parana. In the study by Cao et al.,<sup>(10)</sup> the B\*44 allele (11.7%) was described as more common in Caucasians; a very similar result to the current study (11.1%).

There was a very high frequency of the B\*40 and B\*52 alleles in Asians compared to Caucasians and Afro-Brazilians. In addition, the B\*54 and B\*46 allele were only found in Asians. The high genetic variability between study groups results from the heterogeneous origins and interethnic admixture.

Carnese & Parolin<sup>(16)</sup> studied the genetic variability of different HLA class II alleles characteristic of four Amerindian populations in the region of Patagonia in Argentina and of the Chaco region in Paraguay and identified high frequencies of the DRB1\*04:03, DRB1\*08 and DRB1\*09 alleles. These alleles are extensively distributed throughout the Americas in indigenous populations of North, Central and South America<sup>(16)</sup> and Latin America.<sup>(17)</sup> Some detected allelic variants (DRB1\*04:03, DRB1\*04:05, DRB1\*08:02 and DRB1\*09:01) are widely distributed in Asians, in particular in individuals from Northeastern Asia, but also in Caucasians and African descendants.<sup>(11)</sup> The current study found that the DRB1\*04 allele is widely distributed in Caucasians (12.6%), Afro-Brazilians (9.7%) and especially common in Asians (20.3%) similar to the aforementioned study.<sup>(11)</sup> The DRB1\*08 (11.7%) and DRB1\*09 alleles (12.7%) were most frequently observed in our region in Asians, with a significant difference for the DRB1\*09 allele compared to Caucasians (1.3%) and Afro-Brazilians (1.8%). The results for this locus in this study

confirm those of other studies involving different populations (Chinese, Venezuelan and German).<sup>(9,11,12)</sup>

Regarding the distribution of haplotypes in our region, the HLA-A\*01-B\*08-DRB1\*03 haplotype was significantly more common in Caucasians (2.7%) and Afro-Brazilians (1.5%) as was the HLA-A\*29-B\*44-DRB1\*07 haplotype (Caucasians – 1.64%; Afro-Brazilians – 1.6%). These haplotypes were reported to be more common in Americans descended from Europeans; 4.9% of Americans with Italian ancestry had the HLA-A\*01-B\*08-DRB1\*03 haplotype and 1.5% had the HLA-A\*29-B\*44-DRB1\*07 haplotype. Of Americans with Spanish ancestry, 2.1% had the HLA-A\*01-B\*08-DRB1\*03 haplotype and 2.3% had the HLA-A\*29-B\*44-DRB1\*07 haplotype.<sup>(18)</sup> This study suggests that Spanish Americans are a distinct subgroup of the European American population and more similar to Mexican Americans; Americans of Italian descent are not distinct from the majority of the European American population, especially those from Eastern Europe.<sup>(18)</sup> The estimate that more than one third of the population of the north of Parana State is of Italian descent explains why the data obtained in this study are similar of the genetic pattern of European Americans. The studies by Cao et al.,<sup>(10)</sup> Makhatazde et al.,<sup>(11)</sup> Müller et al.<sup>(12)</sup> and Bicalho et al.<sup>(19)</sup> of populations from North America, Venezuela, Germany and Brazil, respectively corroborate this result. For Asians, the A\*24-B\*52-DRB1\*15 haplotype was the most common (8.2%) different to the results reported by Du et al.<sup>(9)</sup> of a Chinese population, who reported that the A\*02-B\*46-DRB1\*09 haplotype was the most common.

For comparative analysis, the genetic affinity observed in our population in relation to various population groups is possibly due to interethnic gene flow leading to increased intra-population genetic variability. However, this study does not come to any conclusion on the action of evolutionary forces and immigration in the generation and maintenance of the diversity of the HLA system, but gives information on the diversity between groups.

With the obtained demographic data, it was possible to demonstrate the profile of the volunteer bone marrow donor in our region, with greater sensitivity and solidarity shown for bone marrow donation by Caucasians (a finding consistent with data from the IBGE that reports the prevalence of Caucasians in the region) and among young people and women with high school or university education. The characteristics of this profile denote that educational campaigns for public awareness in respect to bone marrow donation have been more effective in this group. Campaigns to register volunteer bone marrow donors have focused largely on schools, universities, industries, shopping centers and regional festivals. There is thus a need to implement educational and awareness campaigns for racial groups that are less well represented in REDOME, in particular Amerindians, Asians and Afro-Brazilians in order to increase the HLA allele frequencies specific to these ethnic groups.

## Conclusions

With the data obtained in this work, there is a need to create bone marrow donation campaigns targeting the ethnic minorities that are underrepresented in REDOME and also focus on different target audiences than that profiled in this study. The results obtained can be used as a reference for INCA/REDOME on the immunogenetic profile of populations in specific regions for the search and selection of bone marrow donors.

Knowledge on the frequency of HLA alleles in our region has led to a better understanding of racial, anthropological and historical aspects of our population, allowing us to compare and track populations from other countries with higher genetic similarity to our population.

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