Human Immunology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

HLA-A, HLA-B and HLA-DRB1 haplotype frequencies in Piauí's volunteer bone marrow donors enrolled at the Brazilian registry

M.G. Carvalho [a], L.T. Tsuneto [b], J.M. Moita Neto [c], L.C.D.M. Sousa [d], H.L.A. Sales Filho [e], M.B. Macêdo [e], J.R.P.M. Barroso [f], E.M. Pereira [d], A.S. Araújo [d], A.S. Silva [d], S.J.H. Monte [d,*]

[a] Master's Program in Science and Health, Federal University of Piauí, Brazil
[b] Maringá State University, Center of Biological Sciences, Department of Basic Health Sciences (DBS), Maringá, Paraná, Brazil
[c] Federal University of Piauí, Chemistry Department, Teresina, Piauí, Brazil
[d] Federal University of Piauí, Laboratory of Immunogenetics and Molecular Biology (LIB), Teresina, Piauí, Brazil
[e] PIBIC – CNPq – Federal University of Piauí, Laboratory of Immunogenetics and Molecular Biology, Teresina, Piauí, Brazil
[f] PIBITI – CNPq – Federal University of Piauí, Teresina, Piauí, Brazil

ARTICLE INFO

Article history:
Received 24 March 2013
Accepted 20 August 2013
Available online xxxx

ABSTRACT

This study aimed to report the antigen and haplotype frequencies (HFs) of volunteer bone marrow donors (VBMDs) from the state of Piauí who were enrolled in the National Volunteer Bone Marrow Donor Registry (REDOME). The research subjects were 21,943 volunteer bone marrow donors, predominantly young adult women (53.3%). The most frequent allelic group was *HLA-A2*, followed by *-DRB1∗13, -DRB1∗04, -DRB1∗07, -B∗15, -B∗35, -B∗44, -A∗24* and *-A∗03*.

Of the 2,704 haplotypes observed, the three most frequent haplotypes were *A∗29 B∗44 DRB1∗07* (1.45%), *A∗01 B∗08 DRB1∗03* (1.4%) and A*03 *B∗07 DRB1∗15* (0.92%). These three haplotypes were in linkage disequilibrium.

PCA showed that 98% of the VBMDs have HLA allele frequencies that are very similar to those from Teresina, the capital city of Piauí. According to the PCA results, these municipalities are distributed with a close proximity to Teresina, which in turn has a close genetic proximity to the Hispanic ethnicity, intermediate proximity to Caucasians and Africans and a distant kinship to Amerindians. The hierarchical proximity of the population of Piauí to the Portuguese and Hispanic populations to shows the strong influence of the latter on the former.

1. Introduction

The transplantation of allogeneic hematopoietic cells is an effective therapy for the treatment of a large number of high-risk blood disorders, but donor shortages are difficult to overcome. The major limitation is finding a suitable match from the pool of donor cells containing highly polymorphic HLA molecules. To expand the number of potential allogeneic hematopoietic cell donors beyond related donors, programs were created that include non-related bone marrow donor registries (BMDRs) around the world.

Bone marrow donor registries were developed in the late 1980s, first in Western countries (Europe and North America) and then in Asian countries (southeast) and Australia. One of the largest BMDRs in South America is the Brazilian program, National Registry of Volunteer Bone Marrow Donors – REDOME. This program was launched in 1993 as a private initiative [1]. In 1998, the Ministry of Health took over the program, and in 2000, it administered the bone marrow transplantation process in Brazil. This arrangement was an important step toward strengthening and growing the registry. As a consequence, REDOME is currently the world's 3rd largest BMDR, and it reached 3,017,036 donors registered by 2012. The huge size of the Brazilian territory, combined with the large amount of genetic diversity observed in the Brazilian population, makes it difficult to develop a BMDR that represents the entire pool of HLAs for a specific Brazilian subpopulation. Thus, to

Abbreviations: BMDR, bone marrow donor registries; BMT, bone marrow transplantation; HCA, hierarchical cluster analysis; HLA, human leukocyte antigen; LD, linkage disequilibrium; NMDP, national marrow donor program; PCA, principal component analysis; PCR-SSOPH, polymerase chain reaction-sequence-specific oligonucleotide probe hybridization; REDOME, national registry of volunteer bone marrow donors; REREME, national registry of bone marrow receptors; VBMD, volunteer bone marrow donors.

* Corresponding author. Fax: +55 86 32155690.
E-mail addresses: marayzacarvalho@yahoo.com.br (M.G. Carvalho), lttsuneto@gmail.com (L.T. Tsuneto), jmoita@ufpi.edu.br (J.M. Moita Neto), claudio.demes@ufpi.edu.br (L.C.D.M. Sousa), hertonluiz@gmail.com (H.L.A. Sales Filho), marinabmacedo@uol.com.br (M.B. Macêdo), jose.renato77@gmail.com (J.R.P.M. Barroso), estermpereira@gmail.com (E.M. Pereira), sousa.analib@gmail.com (A.S. Araújo), adalberto@ufpi.edu.br (A.S. Silva), semiramis@ufpi.edu.br (S.J.H. Monte).

0198-8859/$36.00 - see front matter Crown Copyright © 2013 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humimm.2013.08.283

improve assistance for patients who are on a waiting list for a bone marrow transplant (BMT) from an unrelated donor, it is important to genetically characterize the volunteer bone marrow donors (VBMDs) that are enrolled in REDOME and their regional differences.

Brazil is a continental country that has specific regional characteristics and is peopled by the descendants of immigrants from many countries, who have diverse cultural characteristics and genetic backgrounds. Although there have been studies on the HLA allele frequencies in Brazil, these studies most often assess states that are in the south and southeast [2–4], with relatively few studies on the remaining regions of the country (north, northeast and central-west) [5,6]. One consequence of this disparity in the number of studies is a lack of knowledge about the effective representation of the remaining Brazilian regions in the pool of alleles in the Brazilian population. Thus, our objective is to describe the allele and haplotype frequencies of HLA-A, HLA-B and HLA-DRB1 in the state of Piauí, which is in the Brazilian northeast, and to determine their representation in REDOME.

2. Materials and methods

2.1. Sample

The current study included a sample of VBMDs who were registered at the Laboratory of Immunogenetics and Molecular Biology in Teresina, Piauí, Brazil, from January 2006 to December 2011. The state of Piauí comprises 224 municipalities, with a territorial area of 251,576.644 km^2. It is situated between the geographical coordinates 2°44′ and 10°52′ south latitude and 40°25′ and 45°59′ west longitude. The estimated population of Piauí is 3,118,360 inhabitants, which corresponds to 16.16% of the population of the northeastern region and 2.95% of the entire Brazilian population. All of the donors provided informed consent [7]. The study was approved by the University Research and Ethics Committee.

HLA allelic groups were molecularly typed at an intermediate resolution for the HLA class-I (loci A, B) and class-II (the DRB1 locus) alleles based upon the Polymerase Chain Reaction-Sequence-Specific Oligonucleotide Probe Hybridization (PCR-SSOPH) technique in combination with Luminex using commercial reagents and following the instructions of the manufacturer (One Lambda Inc., Canoga Park, CA, USA). The analysis of the allele genotyping was performed using the HLA Fusion v 1.2.1 Software (One Lambda Inc., Canoga Park, CA, USA). When necessary, allelic groups were converted to the most likely high-resolution types based on the reported HLA frequencies within the observed National Marrow Donor Program (NMDP) multiple allele codes, using the EpHLA converter software (http://ephlaconverter.ufpi.br/) [8].

2.2. Data analysis

Allele frequencies were obtained by direct counting. To calculate the representation of a certain population in REDOME, we used the equation ip = [(state population/Brazil population)/(state VBMDs/REDOME VBMDs)], where "ip" is a proportionality index that evaluates whether the VBMDs from the state are represented in REDOME proportionally to the state's contribution to the country's population. We estimated the maximum-likelihood (ML) haplotype frequencies from the observed data using an Expectation–Maximization (EM) algorithm [9] for multi-locus genotypic data when the gametic phase is not known. Sample haplotype frequencies (hfo) were obtained by an expectation–maximization algorithm. Coefficients of linkage disequilibrium (LD) were obtained by the following formula: D = hfo − hfe, where D is the value of absolute linkage disequilibrium, hfo is the sample haplotype frequency observed and hfe is the expected haplotype frequency, i.e., the product of the frequencies of alleles or variants in the haplotype. For two-point haplotypes, relative delta values were obtained using the equation $\Delta'_{ij} = \Delta_{ij}/|\Delta\max_{ij}|$, where $\Delta\max_{ij}$ takes one of the following values: $\min(pi, pj) - pipj$, if $\Delta_{ij} \geqslant 0$ and $\max(0, pi + pj - 1) - pipj$, if $\Delta_{ij} < 0$ [10]. An exact test was employed to measure the potential deviations from the expected Hardy–Weinberg equilibrium genotype frequencies [11]. The Ewens–Watterson test [12–14] was applied to each locus. The Arlequin software package [15] was used to calculate allele and haplotype frequencies and gene heterozygosity, to verify the fit to Hardy–Weinberg expectations and to perform an Ewens–Watterson's test of selective neutrality.

2.3. Analysis of principal components for genetically characterizing the population

The distribution pattern of the allelic groups in the municipalities of Piauí is a reference for both the grouping and segregation of these subpopulations and for comparison with other populations described in the literature. Thus, we used principal component analysis (PCA) and hierarchical cluster analysis (HCA) (SPSS Software, version 20.0) (Chicago, IL, USA). Briefly, when applied to a multi-dimensional set of variables, PCA calculates an orthogonal basis that is guided by the directions of the maximum variance of the data analyzed. The projections of the original data on this basis, called the *principal components*, accumulate the maximum variance of the data in a decreasing order, thereby making it possible to make an approximate representation of the data from a reduced number of dimensions of the basis. The total variance percentage that is "explained" by a subset of the *principal components* is given by the accumulated sum of the self-values that correspond to that subset of *principal components*.

For the intrapopulation analysis, the municipalities of Piauí with more than 10 VBMD were studied and typed for loci A, B and DRB1 (adding up to 52 municipalities). The allele frequencies for the three loci were tabled for each municipality and were submitted to PCA in comparison with population samples from Brazil – REDOME, REREME [16], REDOME Piauí, REDOME Teresina-PI [17], REDOME Rio Grande do Norte [18], Minas Gerais [19], Paraná (Caucasians, Africans, Mulatto and Cafuzo [a mix of African and Amerindian ancestry] [4]), Terena Indian tribe (Mato Grosso do Sul) [20], United States (Caucasian, Afro-Americans and Hispanic) [21], Portugal (Coimbra, Aveiro and Lisboa), France, Italy, Rwanda [19] and an indigenous population from Peru (Peru Lamas) [22]. Populations with molecular HLA-A, HLA-B and HLA-DRB1 haplotype data, cumulative allele frequencies for each locus that were greater than 90%, and a historical relationship with the genetic makeup of Piauí were included.

The obtained results were used as input parameters to conduct HCA and to group the populations by genetic proximity in a dendrogram using the Jaccard similarity index. The resulting HCA dendrogram consists of diagrams that represent the similarity between sample pairs or groups in a scale that ranges from identity to no similarity.

3. Results and discussion

This study was accomplished with 58.08% (21,943 out of 37,780) [16] of the bone marrow donors from Piauí who were enrolled on REDOME via the Laboratory of Immunogenetics and Molecular Biology of the Federal University of Piauí (LIB-PI). The study subjects were grouped according to their municipalities, which provided three insights the volunteer bone marrow donations in the state of Piauí. The first observation concerns the

Please cite this article in press as: Carvalho MG et al. HLA-A, HLA-B and HLA-DRB1 haplotype frequencies in Piauí's volunteer bone marrow donors enrolled at the Brazilian registry. Hum Immunol (2013), http://dx.doi.org/10.1016/j.humimm.2013.08.283

national representation of Piauí in REDOME. The proportionality index of (ip) = 2 suggests that we should have two times more BMDRs enrolled, so that Piauí is as well-represented on REDOME as the states of São Paulo and Rio Grande do Sul, which showed an ip of 1.1 and 1.04, respectively. The second observation is that there is a differential participation of Piauí municipalities with regard to the volunteer bone marrow donation; of the 224 municipalities of Piauí, only 147 have VBMDs enrolled on REDOME, and from these, 52 (35.4%) show an ip value that is equal to or higher than 10. The third observation concerns the high degree of population admixture found in the studied population, as evidenced by the heterogeneity of the Caucasoid and African alleles and the low contribution of Amerindian genes.

The small representation of Piauí's population on REDOME, together with the high degree of population admixture observed in the state, limits the chances that a bone marrow recipient from Piauí can find a donor in a registry. Here, we showed that the Piauí-VBMDs presented the same demographic profile (Table 1) as the overall profile observed for REDOME in Brazil: predominantly young women [16]. Although at first sight, it appears encouraging to have young people as the main VBMDs in a state, a more realistic interpretation of this scenario could frame it as a concern. This argument is supported by the fact that if it is advantageous for the registry to have a donor with a more lasting donation potential, then it is required that the donor have a strong connection because of being in an age group that has a large migration possibility.

A total of 68 different HLA alleles were found (21 HLA-A, 34 HLA-B and 13 HLA-DRB1). The highest HLA allelic frequencies were observed for the *HLA-A∗02* allelic group (Table 2). The data indicate that there is a greater contribution of the allele groups from European and African origins [23–25], such as *HLA-A∗02*, *HLA-A∗30*, *HLA-B∗15*, *HLA-B∗53*, *HLA-DRB1∗13*, and *HLA-DRB1∗15*. The HLA alleles that are frequently observed in Amerindian populations, such as *HLA-A∗02:04*, *-B∗35:04* and *-B∗35:08*, were not observed in the sample studied, whereas the HLA alleles HLA-*A∗02:11*, *-B∗15:04*, *-DRB1∗04:05*, *-DRB1∗08:07*, *-DRB1∗14:02*, *DRB1∗14:13*, and *-DRB1∗16:02*, which are also typical in Amerindian populations, were observed at frequencies that ranged from 0.0015% to 2.4%.

When the allele frequencies of REDOME-Piauí were compared to those of REDOME Brazil and REREME, a linear regression analysis revealed an r^2 value that was close to 1 (r^2 = 0.9406 for REDOME and r^2 = 0.9681 for REREME); this finding indicates a very high level of correlation between the allele frequencies from Piauí and REDOME Brazil or REREME. Several studies of different markers indicate that the largest amount of genetic variation occurs in individuals who are from the same population and not in individuals who are from different populations [26–29].

With regard to the analysis of haplotype frequencies, 2701 haplotypes were estimated. The most common haplotypes were *A∗29 B∗44 DRB1∗07*, *A∗01 B∗08 DRB1∗03*, *A∗03 B∗07 DRB1∗15*, *A∗02 B∗44 DRB1∗04*, *A∗02 B∗40 DRB1∗04*, *A∗33 B∗14 DRB1∗01*, *A∗02 B∗44 DRB1∗07*, *A∗11 B∗35 DRB1∗01*, *A∗02 B∗15 DRB1∗04* and *A∗02 B∗48 DRB1∗09*.

Hardy–Weinberg exact tests were performed on each of the three loci. We found that the loci HLA-A, -B and -DRB1 were in Hardy–Weinberg equilibrium ($p > 0.05$) with an observed heterozygosity of 89%, 93% and 90%, respectively. The significance of

Table 1
The distribution of VBMDs enrolled in LIB-UFPI REDOME for the period from January 2006 to December 2011 by gender and age.

Age (years)		Gender	
Age group	%	M (%)	F (%)
18–27	38.86	43.94	56.06
28–37	34.31	47.92	52.08
38–47	17.49	45.43	54.57
>48	9.34	43.15	56.85

Table 2
The HLA-A, HLA-B, and HLA-DRB1 allele frequencies in a sample of 21,943 volunteer bone marrow donors from Piauí, Brazil that were REDOME registered in the period from 2006 to 2011.

Alleles *HLA-A∗*	Freq (A) *N* = 21.943	Alleles *HLA-B∗*	Freq (B) *N* = 21.943	Alleles *HLA-DRB1∗*	Freq (DRB1) *N* = 21.943
A∗01	0.0710	*B∗07*	0.0645	*DRB1∗01*	0.0879
A∗02	**0.2548**	*B∗08*	0.0426	*DRB1∗03*	0.0959
A∗03	**0.0788**	*B∗13*	0.0109	***DRB1∗04***	**0.1345**
A∗11	0.0466	*B∗14*	0.0529	***DRB1∗07***	**0.1255**
A∗23	0.0643	***B∗15***	**0.1129**	*DRB1∗08*	0.0716
A∗24	**0.0906**	*B∗18*	0.0362	*DRB1∗09*	0.0221
A∗25	0.0094	*B∗27*	0.0193	*DRB1∗10*	0.0200
A∗26	0.0251	***B∗35***	**0.1088**	*DRB1∗11*	0.0942
A∗29	0.0444	*B∗37*	0.0107	*DRB1∗12*	0.0181
A∗30	0.0687	*B∗38*	0.0178	***DRB1∗13***	**0.1370**
A∗31	0.0646	*B∗39*	0.0380	*DRB1∗14*	0.0475
A∗32	0.0262	*B∗40*	0.0596	*DRB1∗15*	0.0971
A∗33	0.0318	*B∗41*	0.0125	*DRB1∗16*	0.0484
A∗34	0.0102	*B∗42*	0.0201		
A∗36	0.0079	***B∗44***	**0.1014**		
A∗43	0.0000	*B∗45*	0.0193		
A∗66	0.0126	*B∗46*	0.0001		
A∗68	0.0660	*B∗47*	0.0013		
A∗69	0.0020	*B∗48*	0.0115		
A∗74	0.0211	*B∗49*	0.0253		
A∗80	0.0036	*B∗50*	0.0270		
		B∗51	0.0709		
		B∗52	0.0198		
		B∗53	0.0328		
		B∗54	0.0000		
		B∗55	0.0098		
		B∗56	0.0034		
		B∗57	0.0274		
		B∗58	0.0342		
		B∗67	0.0001		
		B∗73	0.0000		
		B∗78	0.0012		
		B∗81	0.0070		
		B∗82	0.0007		

The bold values represent the three most frequent specificities by locus.

Table 3
The Hardy–Weinberg equilibrium test for the HLA-A, HLA-B and HLA-DRB1 Loci in VBMDs from Piauí, Brazil.

Locu	HeObs	HeExp	*p*	SD
HLA-A	0.89199	0.89086	0.08863	0.00014
HLA-B	0.93665	0.93814	0.22861	0.00017
HLA-DRB1	0.90216	0.90122	0.06742	0.00018

HeObs = observed heterozygosity; HeExp = expected heterozygosity, *p* = Hrady-Weiberg signifance, SD = standard deviation. *n* = 21,943.

Table 4
Three-locus HLA haplotype frequencies and linkage disequilibrium.

Haplotype	hfo	Δ	Δ′	$p < 0.0001$
A∗29 B∗44 DRB1∗07	0.0145	0.0140	0.3188	Yes
A∗01 B∗08 DRB1∗03	0.014	0.0137	0.3232	Yes
A∗03 B∗07 DRB1∗15	0.0092	0.0087	0.136	Yes
A∗02 B∗44 DRB1∗04	0.0081	0.0046	0.0475	Yes
A∗02 B∗40 DRB1∗04	0.0081	0.0061	0.1055	Yes
A∗33 B∗14 DRB1∗01	0.0081	0.0079	0.2504	Yes
A∗02 B∗44 DRB1∗07	0.0078	0.0045	0.0461	Yes
A∗11 B∗35 DRB1∗01	0.0071	0.0066	0.1437	Yes
A∗02 B∗15 DRB1∗04	0.0069	0.0030	0.0275	Yes
A∗02 B∗48 DRB1∗09	0.0064	0.0063	0.5566	Yes

hfo: haplotype frequency; Δ: absolute delta value; Δ′: relative linkage disequilibrium; *p*: probability.

Please cite this article in press as: Carvalho MG et al. HLA-A, HLA-B and HLA-DRB1 haplotype frequencies in Piauí's volunteer bone marrow donors enrolled at the Brazilian registry. Hum Immunol (2013), http://dx.doi.org/10.1016/j.humimm.2013.08.283

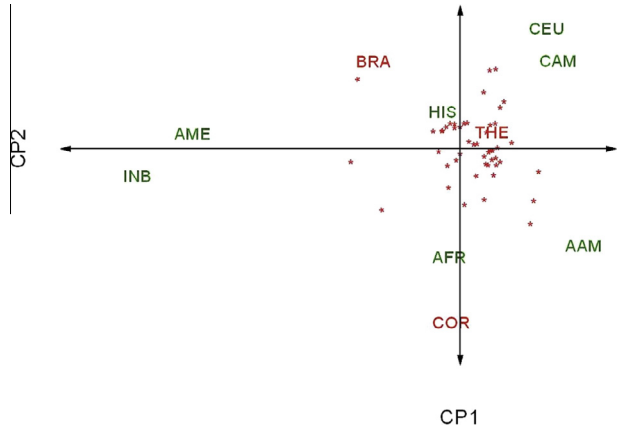

Fig. 1. The distribution of municipalities in Piauí in accordance with the values of the principal components 1 and 2.

the exact test (p), standard deviation, and expected and observed heterozygosity values are shown in Table 3. These results tell us that our population is neither experiencing natural selection nor being mistyped for HLA antigens.

The LD test indicated that there is a possible association between the alleles that make up the haplotypes; the results of this test are shown in Table 4. These results suggest that the strength of the disequilibrium is largely unrelated to the relative physical distance between the loci in this small MHC segment and that functional relatedness has very little, if any, influence on LD. Some possible explanations for this effect include migrations, mutations, racial admixture, new alleles and positive selection. In fact, the migrations of Caucasoid people (from the Brazilian southeast), Portuguese people, and the descendants of African as well as the racial admixture of the latter two ethnic groups are important historical factors in the formation of the people of Piauí. The high frequencies of the former two haplotypes suggest a strong European contribution to the genetic composition of the sample studied.

The low frequencies of HLA alleles and haplotypes of Amerindian origin suggest that the population of Piauí is represented by a pool of genes from primarily Caucasoid, Hispanic and African origins.

For a long time, Amerindians prevailed as the only inhabitants in the state of Piauí. This scenario, however, changed with the arrival of Caucasoid people as from 1571, followed by the Afro-descendants. The immigrants ousted or exterminated the native population of the state and became the predominant people; this is supported by historical population data [30]. The number of Amerindian tribes in Piauí decreased so drastically that the indigenous population of Piauí had essentially disappeared as an ethnic group by the year 1797, according to a report by the President of the province in 1867 [31]. This remains the current situation, as shown by Monte and collaborators in a study of the HLA frequency in people who are born in Piauí (northeastern descendants) [5].

The analysis by principal components emphasizes these previous findings. The first two components comprise the most valuable statistical information of the set on a Cartesian graph, imposing the reduction of the dimensionality of representative points of the 68 original variables. In the principal component analysis, each axis can be separately analyzed. Each point on the graph represents a municipality of Piauí or an ethnic group that is used for compari-

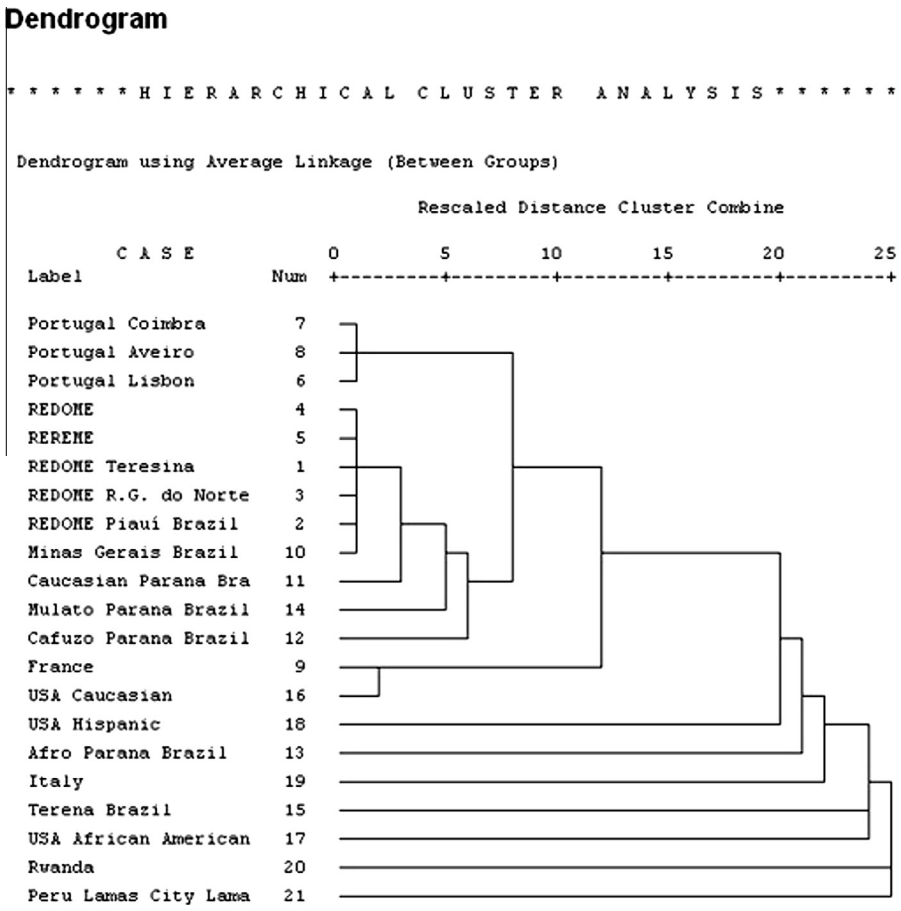

Fig. 2. Hierarchical clustering analysis (HCA) showing the clustering of VBMD from Piauí relative to other Brazilian populations and to other populations around the world. Clustering was performed using PCA output for HLA alleles that are important in discriminating ethnic groups.

Please cite this article in press as: Carvalho MG et al. HLA-A, HLA-B and HLA-DRB1 haplotype frequencies in Piauí's volunteer bone marrow donors enrolled at the Brazilian registry. Hum Immunol (2013), http://dx.doi.org/10.1016/j.humimm.2013.08.283

son (Fig. 1). We assume that the distance between each point on the graph is directly proportional to the genetic distance between the people from the represented municipalities. To improve the visualization of our data and to clarify our results, we highlighted some municipalities and ethnic groups on the graph. This strategy simplified the observation that Teresina (THE) stands out as the center of a group of municipalities that are genetically close to the Hispanic ethnic group (HIS) and further away from the Caucasoid group (Europeans = CEU, and Americans = CAM), Africans (AFR = from Africa, and AAM = from America) and Amerindians (AME and INB). It is interesting to observe that only two municipalities – Brasileira (BRA) (located on the upper part of the graph) and Corrente (COR) (located on the lower part of the graph) – show an intermediate distance to the group of municipalities that are genetically closer to Teresina. The scores for the components showed that the first principal component was influenced by both the low frequency of Amerindian alleles (*HLA-A∗31* and *HLA-DRB1∗14*) and by the intermediate frequency of African origin alleles (*HLA-A∗30* and *HLA-A∗34*). The variations in the second principal component were especially influenced by the low frequency of the *HLA-B∗82* allele, which is a characteristic of Afro-descendants, and by the high frequency of the Caucasoid (*HLA-DRB1∗01, HLA-A∗01*), Hispanic (*HLA-DRB1∗16*) and African (*HLA-B∗57*) alleles.

The principal components analysis may indicate how well a subpopulation genetically represents the population from whence it is derived. It is interesting to note that all of the cases in which the populations are well-represented on REDOME (shown here) indicate a trend in the ip value towards one. Conversely, high ip values are associated with less-represented subpopulations (Brasileira (BRA) – ip of 11.9 and Corrente (COR) – ip of 38.0). Thus, it is reasonable to expect that a decrease in the ip is followed by a simultaneous decrease in the distance between subpopulations of a state and that this difference is easily visualized in the octagonal space circumscribed by the PCA components. If that is true, an increase in the records of volunteer donors from these places would reshape the cluster, which would incorporate these municipalities; therefore, there would not be an HLA phenotype substructure in the population analyzed.

The reliability of the results reported above can be confirmed by comparing our data obtained using HCA (Fig. 2) with the data reported for other populations. The hierarchical proximity of Piauí to the Portuguese (on the top of the tree) and Hispanic (nearer to the base) populations indicates the strong influence of these two populations on the constitution of the people of Piauí. These results corroborate the findings of Pimenta and collaborators, who described the Brazilian population's genetic structure as having 65.9% European influence, 24.8% African influence and 9.3% Amerindian influence [32]. The segregation of the African and Amerindian ethnic groups in our study samples can be explained by the history of the settlement of the Brazilian northeast, where, because of slavery, a stronger influence of Africans and a progressive reduction of the Amerindian population [33] in the Brazilian territory due to the settlement process can be observed [30].

In summary, the current study contributes important information that is potentially usable as a strategy to establish VBMD enrollment policies aimed at forming a database that is representative of the genetic profile of the people of Piauí and that enables individuals to find matched donors with both frequent and uncommon haplotypes.

References

[1] Moraes JR, Moraes ME, Esteves NM, Bendit RK, Romero M, Favrin MA, et al. Brazilian bone marrow bank and registry. Human Immunol 1994;40:114.
[2] Bortolotto AS, Petry MG, da Silveira JG, Raya AR, Fernandes SR, Neumann J, et al. HLA-A, -B, and -DRB1 allelic and haplotypic diversity in a sample of bone marrow volunteer donors from Rio Grande do Sul State, Brazil. Hum Immunol 2012;73:180.
[3] Rosales T, Guilherme L, Chiarella J, Marin ML, Rosales C, Melo CP, et al. Human leukocyte A and B antigen, gene and haplotype frequencies in the population of the city of São Paulo in Brazil. Braz J Med Biol Res 1992;25:39.
[4] Ruiz TM, da Costa SM, Ribas F, Luz PR, Lima SS, Da Graca Bicalho M. Human leukocyte antigen allelic groups and haplotypes in a brazilian sample of volunteer donors for bone marrow transplant in Curitiba, Parana, Brazil. Transplant Proc 2005;37:2293.
[5] Monte SJH, Moita Neto JM, Rampim GF, Shulzhenko N, Morgun A, Gerbase-Delima M. Polimorfismo do sistema HLA em uma amostra de mestiços da população de Teresina, Piauí. Rev Assoc Med Bras 2004;50:422.
[6] Nigam P, Dellalibera E, MaurÎcio-da-Silva L, Donadi EA, Silva RS. Polymorphism of HLA class I genes in the Brazilian population from the northeastern state of Pernambuco corroborates anthropological evidence of its origin. Tissue Antigens 2004;64:204.
[7] IBGE: Pesquisa Nacional por amostra de domicílios e contagem da População – CENSO 2010. Instituto Brasileiro de Geografia e Estatística. 2012.
[8] Barroso JPM. EpHLA Converter. vol 2012. Teresina; 2012.
[9] Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12:921.
[10] Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethni groups. In: Tsuji K, Aizawa M, Sasazuki T, editors. HLA 1991 proceedings of the eleventh international histocompatibility workshop and conference. New York: Oxford Science; 1992. p. 1065.
[11] Guo SW, Thompson EA. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 1992;48:361.
[12] Watterson GA. The homozygosity test of neutrality. Genetics 1978;88:405.
[13] Hartl DL, Clark AG. Principles of population genetics. 3rd. Sunderland: Sinauer Associates, Inc.; 1997.
[14] Ewens WJ. The sampling theory of selectively neutral alleles. Theor Popul Biol 1972;3:87.
[15] Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinform Online 2005;1:47.
[16] Bouzas LFS. Análise da capacidade do REDOME/RENACORD em suprir as necessidades dos pacientes registrados no REREME. Instintuto Nacional do Câncer, vol Doutorado. Rio de Janeiro: Programa de Pósgraduação em Oncologia/INCA; 2011 (p 91).
[17] Carvalho MG, Sales Filho HLA, Macêdo MB, Barroso JRPM, Sousa LCDM, Araújo AS, et al. Distribuição de Alelos e Haplótipos de Antígenos Leucocitários Humanos Loci A, B e DRB1 em uma amostra de Doadores Voluntários de Medula Óssea (DVMO) do Piauí. Rev Bras Hematol Hemoter 2012;34:17.
[18] Macêdo MB, Hemonorte, Carvalho MG, Moita Neto JMM, Tsuneto LT, Silva AS, et al. Distribuição de Alelos e Haplótipos de Antígenos Leucocitários Humanos Loci A, B e DRB1 em uma amostra de Doadores Voluntários de Medula Óssea (DVMO) do Rio Grande do Norte. Rev Bras Hematol Hemoter 2012;34:19.
[19] Allele∗Frequencies in Worldwide Populations The Allele Frequency Net Database, 2013.
[20] Lazaro AM, Moraes ME, Marcos CY, Moraes JR, Fernandez-Vina MA, Stastny P. Evolution of HLA-class I compared to HLA-class II polymorphism in Terena, a South-American Indian tribe. Hum Immunol 1999;60:1138.
[21] Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. Hum Immunol 2007;68:779.
[22] Moscoso J, Seclen S, Serrano-Vela JI, Villena A, Martinez-Laso J, Zamora J, et al. HLA genes in Lamas Peruvian-Amazonian Amerindians. Mol Immunol 2006;43:1881.
[23] Ribas F, Oliveira LA, Petzl-Erler ML, Bicalho MG. Major histocompatibility complex class I chain-related gene A polymorphism and linkage disequilibrium with HLA-B alleles in Euro-Brazilians. Tissue Antigens 2008;72:532.
[24] Piazza A, Olivetti E, Griffo RM. The distribution of HLA antigens in Italy. Genes Geogr 1989;3:141.
[25] Carvalho AS. HLA-A, -B and -C markers in the Portuguese population. Tissue Antigens 1983;21:39.
[26] Lewortin RC. The genetic basis of evolutionary change. New York: Columbia University Press; 1974.
[27] Barbujani G, Magagni A, Minch E, Cavalli-Sforza LL. An apportionment of human DNA diversity. Proc Natl Acad Sci USA 1997;94:4516.
[28] Roseberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK. Clines, cluster, and the effect of study design on the inference of human population structure. PLoS Genet 2005;1:70.
[29] Wang S, Lewis CM, Jakobsson M, Ramachandran S, Ray N, Bedoya G, et al. Genetic variation and population structure in native Americans. PLoS Genet 2007;3:e185.
[30] Dias CC. PYAUHY das origens à nova Capital. 2nd ed. Nova Expansão Gráfica e editora Ltda; 2009.
[31] Brandão TMP. A Elite Colonial Piauiense: Família e Poder. Teresina: Fundação Cultural Monsenhor Chaves; 1995.
[32] Pimenta JR, Zuccherato LW, Debes AA, Maselli L, Soares RP, Moura-Neto RS. Color and genomic ancestry in Brazilians: a study with forensic microsatellites. Hum Hered 2006;62:190.
[33] Pena SDJ, Bastos-Rodrigues L, Pimenta JR, Bydlowski SP. DNA tests probe the genomic ancestry of Brazilians. Braz J Med Biol Res 2009;42:870.

Please cite this article in press as: Carvalho MG et al. HLA-A, HLA-B and HLA-DRB1 haplotype frequencies in Piauí's volunteer bone marrow donors enrolled at the Brazilian registry. Hum Immunol (2013), http://dx.doi.org/10.1016/j.humimm.2013.08.283