Human Platelet Antigens in Brazilian Multiethnic Populations: Occurrence of Regional Variation and Frequency in a Large Urban Center (Belo Horizonte)

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Keywords

Platelets · Human platelet antigens · Real-time PCR · Genotyping

Summary

Background: The frequency of human platelet antigens (HPA) varies according to ethnicity, which causes differences in the morbidity of alloimmune and autoimmune thrombocytopenic disorders in different populations. Studies on HPA frequencies in Brazil have reported differences among Brazilian populations produced by the diverse degrees of admixture throughout the country.

Methods: In the present study, we investigated the variation of HPA distribution in Brazil, compared with worldwide populations, and describe the frequencies of HPA-1, -2, -3, -5, and -15 in a large urban center in Southern Brazil (Belo Horizonte) based on a sample of blood donors. **Results:** The principal component analysis and the dendrogram based on genetic distance revealed a clear relationship between Brazilian populations and the groups formed by European and African populations. The coefficients of variation for HPA allele frequencies suggest that Brazilian populations presented variations for HPA alleles comparable with the populations from continental groups. In Belo Horizonte, the allele frequencies for HPA-1, -2, -3, -5 and -15 were 0.8575, 0.8400, 0.6225, 0.8525 and 0.5825 respectively. The genotypes with higher frequencies were a/a (72–74%), except for HPA-3 and -15, whose heterozygous a/b genotypes were shown to be more prevalent (43.5 and 44.5%, respectively). **Conclusion:** We confirmed the heterogeneity of HPA antigens in Brazilian populations, reinforcing the importance of HPA panels composed of regional blood

donors, or a national panel that contemplates the specificities of the different regions of the country, in order to provide support in platelet transfusions and to minimize the risks associated with HPA alloimmunization. The evaluation of HPA data from Belo Horizonte represents the initial step toward the development of a genotyped platelet donor registry in order to treat HPA alloimmunized patients in this region.

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Introduction

Platelet transfusions are administered to prevent or treat bleeding in patients with quantitative or qualitative platelet disorders. However, human platelet antigens (HPA) can induce alloantibody production after HPA-incompatible transfusion in antigen-negative individuals or due to fetomaternal incompatibility for HPA [1]. Alloantibodies against HPA are implicated in alloimmune thrombocytopenic disorders (AIT), like post-transfusion purpura or neonatal alloimmune thrombocytopenia (NAIT), and in refractoriness to platelet transfusions [1–4].

To date, the Immuno Polymorphism Database lists 35 platelet alloantigens (www.ebi.ac.uk/ipd/hpa/). The frequency of these antigens varies according to ethnicity, which causes differences in the incidence of antigen specificity of anti-HPA antibodies and in the morbidity of AIT in different populations [5, 6]. In Europeans, alloantibodies are formed most commonly against HPA-1, -2, and -5. Antibodies against HPA-1a account for more than 80% of cases of NAIT in Africans and Europeans [4]. In contrast, alloimmunization to anti-HPA-4b is the predominant cause of NAIT in the Japanese population [7].

Table 1. Populations included in the comparisons of HPA frequencies

Ancestry	Worldwide populations	
	populations from previous studies	populations from the 1000 Genomes Project Consortium [12]
African	4- Benin, Fon, Adja [13] 18- Cameroon, Bamileke, Duala, Beti [13] 19- Central African Republic, Aka pygmies [13] 22- Congo, Bantu [13]	30- African Caribbean in Barbados 31- African Ancestry in Southwest US 32- Esan in Nigeria 33- Gambian in Western Division 34- Luhya in Webuye, Kenya 35- Mende in Sierra Leone 36- Yoruba in Ibadan, Nigeria
Amerindian	2- Argentinian Amerindian Toba [14] 7- Brazil, Amazon Indians [10] 10- Amazon Indians (Xikrin-Kayapo) [15]	37- Colombian in Medellin, Colombia 38- Mexican Ancestry in Los Angeles 39- Peruvian in Lima, Peru 40- Puerto Rican in Puerto Rico
East Asia	20- China, Han [16] 21- China, Han [17] 29- Vietnam, Kinh [18] 24- Ma'ohis_French Polinesia [18]	41- Chinese Dai in Xishuangbanna, China 42- Han Chinese in Beijing, China 43- Southern Han Chinese, China 44- Japanese in Tokyo, Japan 45- Kinh in HoChiMinh City, Vietnam
European	1- Argentinian Caucasian [14] 3- Austria/Styria, Caucasian [19] 23- Czech Republic, Caucasian [20] 25- Germany, Caucasians [21] 26- Italy, Caucasian [22] 27- Slovenian, Caucasian [23] 28- Switzerland, Caucasian [24]	46- Utah, Northern, and Western European Ancestry 47- Finnish in Finland 48- British in England and Scotland 49- Iberian populations in Spain 50- Toscani in Italy
South Asia		51- Bengali in Bangladesh 52- Guraji Indian in Houston, TX 53- Indian Telugu in the UK 54- Punjabi in Lahone, Pakistan 55- Sri Lanka Tamil in the UK
Ancestry	Brazilian populations	
Brazil	5- São Paulo – Caucasian [10] 6- Bahia – African-descents [10] 8- Paraná – blood donors [11] 9- Paraná – blood donors – Japanese descent [11] 11- São Paulo – blood donors [15] 12- Amazonas – blood donors [25] 13- Rio Grande do Sul – platelet donors – Caucasian [26] 14- Rio Grande do Sul – platelet donors – non Caucasian [26] 15- São Paulo – platelet donors [5] 16- São Paulo – patients with hematologic malignancies [27] 17- Minas Gerais – platelet donors (present study)	

HPA determination is important for the diagnosis and treatment of patients with AIT syndromes. Platelet genotyping is considered the gold standard for HPA typing and is useful when correlated with the results of antibody testing to determine alloimmunization and to measure the risk of AIT [8].

The Brazilian population is very heterogeneous, being formed by extensive admixture between Amerindians, Africans, and Europeans [9]. Studies on HPA frequencies in Brazil have proposed the occurrence of heterogeneity across the Brazilian population produced by the different degrees of admixture throughout the coun-

try, suggesting the importance of the formation of HPA panels composed of regional blood donors [10, 11].

In this study we present a broad comparison of HPA in worldwide populations, including data from Brazilian populations from different regions and with different ethnic backgrounds. Furthermore, we describe for the first time the frequencies of HPA-1, -2, -3, -5, and -15 in Belo Horizonte, a large urban center in Southern Brazil, based on a sample of blood donors. Belo Horizonte is the capital of the State of Minas Gerais, located in the southeast region of Brazil, and its metropolitan area (9,460 km² and approximately

5 million inhabitants) is ranked as the third most populous area in Brazil. The genotype data integrated a nascent database for searching for HPA-compatible donors in order to treat HPA-alloimmunized patients in this region.

These results provide an informative background of HPA polymorphisms in this highly admixed population and may be useful to support platelet transfusions, minimizing the risks associated with HPA alloimmunization.

Material and Methods

Study Population

In total, 200 volunteer platelet apheresis donors participated in the study. All individuals were male and unrelated to each other. The sample consisted exclusively of male donors because the Fundação Hemominas selects only men for apheresis platelet donations. This measure is taken to reduce the risk of TRALI since women are more likely to produce anti-HLA antibodies as a result of pregnancy. However, since it is expected that there is no difference in the frequency of HPA between men and women, we consider that this sample represents the general population. The donors were enrolled between March and May 2016 at Belo Horizonte blood center (Hemocentro de Belo Horizonte, Fundação Hemominas). The Fundação Hemominas is the main public blood

center in Belo Horizonte. Written consent for the study was obtained from all individuals.

HPA frequency data from worldwide populations used for analysis and comparisons were obtained from the Immuno Polymorphism Database, EMBLEBI (www.ebi.ac.uk/ipd/hpa/index.html), the 1000 Genomes project (www.internationalgenome.org/) [12], or were derived from previous studies on HPA frequencies (tables 1, 2). Only populations with results for at least HPA-1, 2, 3, and 5 and with data published in scientific studies were included in our analysis.

This study was approved by the Hemominas Ethical Committee (CAAE 43237615.7.0000.5118).

HPA Genotyping

Genomic DNA was extracted from the peripheral blood samples of platelet donors using the Biopur Kit (Mobius Life Science, Pinhais, Brazil), according to the manufacturer's instructions. Genotyping for HPA-1 (rs 5918), -2 (rs6065), -3 (rs5911), -5 (rs1801106), and -15 (rs10455097) used 1× TaqMan™ SNP genotyping assay (Applied Biosystems, Foster City, CA, USA), 1× TaqMan™ Genotyping Master Mix (Applied Biosystems), and 100 ng DNA. Each pre-designed TaqMan™ SNP genotyping assay (C__818008_30, C__11442703_10, C__3017440_10, C__27862812_10 and C__3226894_10 for HPA-1, -2, -3, -5, and 15, respectively) contained VIC and FAM dye-labeled probes for the alleles a and b and two target-specific primers. HPA-1, -2, -3, -5, and 15 were selected among the more than 30 known HPA due to their relevance in transfusion medicine. All reactions were performed on a real-time PCR system model ABI 7500 Fast (Applied Biosystems) under the following conditions: a pre-amplifi-

Table 2. Human platelet antigens alleles and genotypes in Brazil compared to worldwide populations

N	Population	HPA allele "a" frequencies					Reference
		HPA-1	HPA-2	HPA-3	HPA-5	HPA-15	
1	Argentinian Caucasian	0.878	0.875	0.612	0.927	0.511	[14]
2	Argentinian Amerindian Toba	1.000	0.944	0.389	1.000	0.685	[14]
3	Austria/Styria, Caucasian	0.830	0.870	0.620	0.920	NA	[19]
4	Benin, Fon, Adja	0.896	0.708	0.679	0.818	0.646	[13]
5	Brazil, Caucasian	0.925	0.850	0.600	0.920	NA	[10]
6	Brazil, African-descents	0.903	0.810	0.666	0.876	NA	[10]
7	Brazil, Amazon Indians	1.000	0.821	0.757	1.000	NA	[10]
8	Brazil, mixed blood donors (Paraná)	0.881	0.884	0.659	0.925	0.522	[11]
9	Brazil, Japanese descents (Paraná)	0.978	0.893	0.579	0.975	0.553	[11]
10	Amazon Indians (Xikrin-Kayapo)	1.000	0.963	0.708	0.963	0.780	[15]
11	Brazil, blood donors (São Paulo)	0.918	0.816	0.640	0.825	NA	[15]
12	Brazil, blood donors (Amazonas)	0.862	0.852	0.665	0.892	0.502	[25]
13	Brazil, blood donors (Porto Alegre), Caucasian	0.867	0.895	0.621	0.903	0.500	[26]
14	Brazil, blood donors (Porto Alegre), non-Caucasian	0.794	0.868	0.662	0.809	0.471	[26]
15	Brazil, blood donors (São Paulo)	0.853	0.878	0.649	0.902	0.533	[5]
16	Patients with hematologic malignancies (São Paulo)	0.837	0.830	0.700	0.887	0.457	[27]
17	Brazilian blood donors (Belo Horizonte)	0.858	0.840	0.623	0.853	0.583	present study
18	Cameroon, Bamileke, Duala, Beti	0.907	0.763	0.614	0.746	0.691	[13]
19	Central African Republic, Aka pygmies	1.000	0.607	0.5	0.595	0.698	[13]
20	China, Han	0.994	0.952	0.5945	0.986	0.532	[16]
21	China, Han	1.000	0.955	0.588	0.977	0.582	[17]
22	Congo, Bantu	0.904	0.776	0.596	0.732	0.701	[13]
23	Czech Republic, Caucasian	0.830	0.90	0.59	0.93	NA	[20]
24	Ma'ohis_Frensh Polinesia	0.975	0.913	0.599	0.975	0.463	[18]
25	Germany, Caucasians	0.839	0.910	0.586	0.917	NA	[21]
26	Italy, Caucasian	0.850	0.89	0.61	0.9	NA	[22]
27	Slovenian, Caucasian	0.832	0.899	0.667	0.892	NA	[23]
28	Switzerland, Caucasian	0.809	0.918	0.591	0.934	NA	[24]
29	Vietnam, Kinh	0.986	0.953	0.486	0.972	0.477	[H18]

Table 2 continued on next page

Table 2. Continued

N	Population	HPA allele "a" frequencies					Reference
		HPA-1	HPA-2	HPA-3	HPA-5	HPA-15	
30	ACB: African Caribbean in barbados	0.891	0.755	0.562	0.797	0.365	The 1000 Genomes Project Consortium
31	ASW: African Ancestry in Southwest US	0.918	0.754	0.574	0.803	0.377	
32	ESN: Esan in Nigeria	0.909	0.793	0.646	0.783	0.359	
33	GWD: Gambian in Western Division	0.929	0.774	0.527	0.898	0.358	
34	LWK: Luhya in Webuye, Kenya	0.884	0.692	0.606	0.636	0.318	
35	MSL: Mende in Sierra Leone	0.982	0.712	0.494	0.841	0.341	
36	YRI: Yoruba in Ibadan, Nigeria	0.861	0.755	0.556	0.810	0.310	
37	CLM: Colombian in Medellin, Colombia	0.894	0.856	0.644	0.920	0.548	
38	MXL: Mexican Ancestry in Los Angeles, CA	0.898	0.867	0.570	0.961	0.367	
39	PEL: Peruvian in Lima, Peru	0.947	0.818	0.594	0.959	0.524	
40	PUR: Puerto Rican in Puerto Rico	0.861	0.846	0.702	0.909	0.495	
41	CDX: Chinese Dai in Xishuangbanna, China	1.000	0.935	0.484	0.957	0.452	
42	CHB: Han Chinese in Beijing, China	0.990	0.937	0.544	0.981	0.495	
43	CHS: Southern Han Chinese, China	0.995	0.967	0.576	0.971	0.429	
44	JPT: Japanese in Tokyo, Japan	0.990	0.856	0.601	0.947	0.538	
45	KHV: Kinh in HoChiMinh City, Vietnam	0.980	0.955	0.505	0.985	0.449	
46	CEU: Utah, Northern and Western European Ancestry	0.874	0.944	0.626	0.899	0.429	
47	FIN: Finnish in Finland	0.889	0.864	0.571	0.924	0.646	
48	GBR: British in England and Scotland	0.868	0.934	0.621	0.934	0.478	
49	IBS: Iberian populations in Spain	0.864	0.907	0.603	0.893	0.467	
50	TSI: Toscani in Italy	0.846	0.916	0.636	0.911	0.505	
51	BEB: Bengali in Bangladesh	0.890	0.930	0.628	0.901	0.651	
52	GIH: Guraji Indian in Houston, TX	0.908	0.932	0.660	0.922	0.680	
53	ITU: Indian Telugu in the UK	0.877	0.936	0.672	0.907	0.686	
54	PJL: Punjabi in Lahore, Pakistan	0.870	0.932	0.656	0.932	0.615	
55	STU: Sri Lanka Tamil in the UK	0.887	0.956	0.701	0.897	0.652	

cation step of 60 °C for 30 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 64 °C for 1 min, and a post-amplification step of 60 °C for 30 s. We successfully constructed positive controls for use in the reactions, containing the specific SNP of each allele in four different fragment sequences (gBlocks™ Gene Fragments; Integrated DNA Technologies, Skokie, IL, USA): HPA-1a-2a; HPA-1b-2b, HPA-3a-5a-15b, and HPA-3b-5b-15a. The analysis was performed using the Allelic Discrimination Plot.

Statistical Analysis

The HPA genotype and allele frequencies were compared according to ABO and Rh blood group systems by chi square or Fisher's exact test using the GraphPad Prism 5 software package (GraphPad Software, San Diego, CA, USA). Tests of Hardy-Weinberg equilibrium were performed for each HPA comparing expected and observed genotype frequencies by Fisher's exact test. Differences were considered significant when p values were <0.05.

Principal component analysis (PCA) based on the HPA-1, -2, -3, -5, and -15 allele frequencies was performed by using the ClustVis web tool [28] to analyze the relationship between Belo Horizonte blood donors, individuals from other Brazilian regions, and the worldwide populations. Table 1 shows the populations used in this analysis.

Genetic distances based on the HPA alleles were used to construct a dendrogram using the neighbor-joining method implemented in the PHYLIP software package [29, 30] to summarize the affinities between the Brazilian and other populations. The tree was drawn with the FIGTREE program (<http://tree.bio.ed.ac.uk/software/figtree/>). The worldwide populations used in this analysis belong to 1000 Genomes projects, and the Brazilian populations were derived from previous studies, with the exception of Belo Horizonte (table 1). Populations from the 1000 Genomes project were selected because this dataset provides a reliable source of information about human genetic variation generated

based on whole genome sequencing data from more than 2,500 individuals across 26 populations from five continental groups [12].

The coefficient of variation (CV) of HPA allele frequencies was calculated as the ratio of the standard deviation to the mean for the five continental population groups characterized as part of the 1000 Genomes Project and for the Brazilian populations.

Comparisons of HPA allele count between different Brazilian groups were assessed by the chi square test using the OpenEpi tool (www.openepi.com/RbyC/RbyC.htm). A p value < 0.05 was considered a statistically significant difference.

Results

HPA genotyping was performed in 200 male platelet apheresis donors with mean blood donations of 21 (range 2–139) and a mean age of 38 years (range 19–61 years). Their allele and genotype frequencies are shown in table 3. The allele frequencies of all analyzed HPA systems were in Hardy-Weinberg equilibrium ($p > 0.05$). The genotypes with higher frequencies were a/a (72–74%), except for HPA-3 and -15, whose heterozygous a/b genotypes were shown to be more prevalent (43.5% and 44.5%, respectively). The most common genotype was HPA-1a/a (74%), followed by HPA-5a/a (72.5%) and HPA-2a/a (72%), whereas the most uncommon was HPA-5b/b (2%), followed by HPA-1b/b (2.5%).

We also analyzed the frequency of HPA genotyping according to ABO and Rh blood systems. The most frequent blood group in

Table 3. HPA allele and genotype frequencies in blood donors from Belo Horizonte, Brazil

Gene	Allele		Genotype		
	a	b	a/a	a/b	b/b
HPA-1	0.8575	0.1425	148 (0.740)	47 (0.235)	5 (0.250)
HPA-2	0.8400	0.1600	144 (0.720)	48 (0.240)	8 (0.040)
HPA-3	0.6225	0.3775	81 (0.405)	87 (0.435)	32 (0.160)
HPA-5	0.8525	0.1475	145 (0.725)	51 (0.255)	4 (0.020)
HPA-15	0.5825	0.4175	72 (0.360)	89 (0.445)	39 (0.195)

Table 4. HPA genotypes according ABO and Rh blood groups

HPA genotype	ABO/RhD blood group					
	O/RhD+	A/RhD+	B/RhD+	AB/RhD+	O/RhD–	B/RhD–
HPA-1						
aa	77 (73.33%)	44 (69.84%)	10 (71.43%)	5 (100%)	12 (100%)	0
ab	25 (23.81%)	18 (28.57%)	3 (21.43%)	0	0	1 (100%)
bb	3 (2.86%)	1 (1.59%)	1 (7.14%)	0	0	0
HPA-2						
aa	79 (75.24%)	42 (66.67%)	10 (71.43%)	2 (40.00%)	10 (83.33%)	1 (100%)
ab	22 (20.95%)	17 (26.98%)	4 (28.57%)	3 (60.00%)	2 (16.67%)	0
bb	4 (3.81%)	4 (6.35%)	0	0	0	0
HPA-3						
aa	42 (40.00%)	24 (38.09%)	6 (42.86%)	2 (40.00%)	6 (50.00%)	1 (100%)
ab	46 (43.81%)	27 (42.86%)	7 (50.00%)	2 (40.00%)	5 (41.67%)	0
bb	17 (16.19%)	12 (19.05%)	1 (7.14%)	1 (20.00%)	1 (8.33%)	0
HPA-5						
aa	75 (71.43%)	46 (73.02%)	11 (78.57%)	4 (80.00%)	9 (75.00%)	0
ab	26 (24.76%)	17 (26.98%)	3 (21.43%)	1 (20.00%)	3 (25.00%)	1 (100%)
bb	4 (3.81%)	0	0	0	0	0
HPA-15						
aa	35 (33.33%)	25 (39.68%)	6 (42.86%)	2 (40.00%)	3 (25.00%)	1 (100%)
ab	54 (51.43%)	20 (31.75%)	5 (35.71%)	2 (40.00%)	8 (66.67%)	0
bb	16 (15.24%)	18 (28.57%)	3 (21.43%)	1 (20.00%)	1 (8.33%)	0

the randomly enrolled donors was O+ (52.5%) followed by A+ (31.5%) (table 4). Comparing these two larger groups, the HPA genotype frequency was not significantly different, except for HPA-15 (chi square, $p = 0.025$), whose A+ blood donors had a smaller proportion (31.75%) of heterozygous than O+ individuals (51.43%). On the other hand, the allelic frequency was not significantly different between A+ and O+ individuals for any of the HPA alleles.

We identified 67 different HPA combinations, 31 of which occurred in only one individual, evidencing the diversity of allelic combinations. The most frequent was HPA-1aa/-2aa/-3ab/-5aa/-15ab (17/200, 8.5%). 20 (10%) donors were homozygous a/a for HPA-1, -2, -3, -5, and heterozygous a/b ($n = 13$) or homozygous b/b ($n = 7$) for HPA-15. In addition, 13 (6.5%) platelet donors were b-negative (homozygous a/a) for all HPA analyzed. As expected, individuals simultaneously heterozygous a/b or homozygous b/b for all HPA were not found. We also identified 13 individuals carrying rare allele combinations: HPA-3bb/15bb (3.5%), HPA-2bb/15bb (2.5%), and HPA-3bb/5bb (0.5%). Other combinations

of homozygous b/b were not found. Four (2%) donors (O+: 2; A+: 1; B+: 1) had the genotypic pattern HPA-1a/5b-negative (HPA-1bb/5aa), which is the most compatible profile to treat neonatal alloimmune thrombocytopenia.

A PCA was performed (fig. 1) based on the HPA-1, -2, -3, -5, and -15 allele frequencies using the ClustVis web tool and considering the populations listed in table 1. To facilitate the analysis, we labeled the populations as follows: African, European, East Asia, South Asia, and Amerindian according to the geographic or ethnic origin (when the original study described the population as belonging to a particular ethnic group). Brazilian populations were labeled 'Brazil,' with the exception of Native American populations, which were labeled 'Amerindians.' PCA showed that the first two principal components accounted for 69.1% of the variability (39% along axis 1 and 30.1% along axis 2), and revealed a clear relationship between diversity pattern of HPA and geographical/ethnic origin (fig. 1). In this analysis, the Brazilian populations were distributed heterogeneously, mainly between the groups formed by European and African populations.

Fig. 1. PCA based on the gene frequencies of HPA-1, -2, -3, -5, and -15 showing the relationships among different Brazilian and worldwide populations. The populations are described in table 1.

Fig. 2. Dendrogram based on HPA-1, -2, -3, and -5 frequencies showing the genetic relationships among Brazilians and other worldwide populations. The populations are presented in table 1.

To evaluate the variation in HPA allelic frequencies in Brazil in comparison with other groups, we calculated the CV of HPA allele frequencies for the Brazilian populations and for the populations that integrated each of the five continental groups from the 1000 Genomes Project. In the case of Brazilian populations, the CV was calculated considering all populations that originated from Brazil (populations 5 to 17, table 1), and considering exclusively the predominantly admixed populations characteristic of the different

Brazilian regions (populations 8, 11, 12, 15, 16, and 17, table 1). The CV calculated for populations from continental groups ranged from 0.01 to 0.04 for HPA-1, 0.01 to 0.05 for HPA-2, 0.04 to 0.09 for HPA-3, 0.02 to 0.10 for HPA-5, and 0.04 to 0.17 for HPA-15 (table 5). In general, the CVs of Brazilian populations were within the range of CVs observed for the world's populations.

Comparison of HPA alleles between Brazilian populations was assessed by a global chi square test. Statistically significant differ-

ences were observed for allele distributions of HPA-1, -2, -3, -5, and 15 when all Brazilian populations were considered ($p < 0.01$). However, when only predominantly admixed Brazilian groups were included in this analysis, a significant difference was observed only for HPA-5 and -15 ($p = 0.004$ and 0.019 , respectively).

The heterogeneity in the HPA allelic frequency observed among distinct Brazilian populations, as reported in previous studies, was confirmed and expanded by these analyses, with the simultaneous inclusion of populations living in almost all Brazilian geographic regions: north, northeast, south, and southeast. Only the center-west region was not included due to the absence of data.

A dendrogram was constructed using a genetic distance matrix combining HPA-1, -2, -3, and, -5 loci (fig. 2). In this dendrogram, only data from Brazil or the 1000 Genomes project were included in order to provide a more clear and informative tree. HPA 15 was not included in this analysis due to the large number of missing data for Brazilian populations. In this dendrogram, the majority of Brazilian admixed populations, including blood donors from Belo Horizonte, were included in a branch that contained African populations.

Discussion

Several studies have reported the distributions of HPA polymorphisms in worldwide populations, showing the existence of great variation among different ethnic and geographic groups. The Brazilian population is highly heterogeneous and admixed, presenting important differences in genomic ancestry among regions [9, 31]. Studies carried out in Brazilian populations have demonstrated the existence of significant differences in the distribution of HPA antigens throughout the Brazilian territory [10, 25], which could generate regional differences in the risk of HPA alloimmunization and its consequences. In the present study, we investigated the variation of HPA frequencies in different Brazilian populations, compared with other world populations.

PCAs, performed with the HPA-1, -2, -3, -5, and -15 allele frequencies showed that, worldwide, populations from the same ethnic or geographic region tended to group together, revealing, as expected, affinity for the platelet antigen profile. Only the populations labeled as Amerindians (populations 2, 7, 10, 37, 38, 39, and 40) did not form a reasonably well-defined cluster. This result can be explained by the existence of marked differences in the frequency of platelet antigens among Amerindian populations, which have been attributed to the enormous amounts of genetic drift present in many South American native groups that has generated exceptional allele frequency variation [32, 33].

In PCA, Brazilian populations were distributed heterogeneously among European and African populations. This pattern is in agreement with historical records of the Brazilian colonization, corroborated by genetic studies. The Brazilian population originated from three main ancestral contributions: African, European, and Native American. However, their genetic ancestry varies greatly among the different Brazilian regions, with the largest African ancestry in northeastern areas, predominant European ancestry in the south-

Table 5. Coefficient of variation for HPA allele frequencies in different populations

Population*	Coefficient of variation for HPA allele 'a' frequencies				
	HPA-1	HPA-2	HPA-3	HPA-5	HPA-15
African	0.04	0.05	0.09	0.10	0.07
Native American	0.04	0.02	0.09	0.03	0.17
East Asia	0.01	0.05	0.09	0.02	0.09
European	0.02	0.03	0.04	0.02	0.17
South Asia	0.02	0.01	0.04	0.02	0.04
Brazil (all)*	0.07	0.05	0.07	0.06	0.18
Brazil (admixed) [§]	0.03	0.03	0.04	0.04	0.09

*Worldwide populations belong to 1000 Genomes projects; Brazilian populations were derived from previous published studies.

[§]Brazil (all): populations originated from Brazil including native American populations and groups artificially selected based on ethnic characteristics (populations 5 to 17 in table 1).

[§]Brazil (admixed) admixed populations from different Brazilian regions (populations 8, 11, 12, 15, 16 and 17 in table 1).

eastern and southern areas, and a larger influence of the Amerindian root in northern Brazil. Additionally, a great variation in individual admixture has been observed in Brazil [9, 31]. Blood donors from Belo Horizonte were consistently grouped with other Brazilian populations, reflecting the ancestry observed in blood donors from Minas Gerais, previously estimated as 33.8% African, 57.7% European, and 3.5% Amerindian [34].

The PCA (fig. 1) and the dendrogram constructed using a genetic distance matrix combining HPA loci (fig. 2) also revealed a very close relationship between some Brazilian groups and worldwide populations, such as population 13 [26] and the European populations, and population 9 [11] and East Asian populations. However, it is important to note that these are not natural populations but rather groups of individuals selected by ethnic-racial criteria and described as 'Caucasians' (population 13) or 'Japanese descendants' (population 9).

To analyze the heterogeneity of HPA distribution in Brazil, we compared the CVs for HPA allele frequencies for populations from the five continental groups that were part of the 1000 Genomes Project and for Brazilian populations. The comparison indicates that, in general, Brazilian populations presented variation for HPA alleles comparable to the populations from continental groups. This occurred even when only admixed Brazilian populations were considered, although, in this case, the CVs were smaller (table 5). These results suggest that the frequencies of HPA observed in the different Brazilian populations may be as variable as those observed between the different countries of a continent. On the other hand, attention must be paid to the inclusion of artificially selected groups based on ethnic characteristics in this analysis to avoid overestimating the heterogeneity of HPA distribution. This observation was corroborated by the global chi square test performed for the distribution of HPA alleles in Brazilian populations.

The development of a panel of HPA-genotyped donors is important to enable the matched platelet selection for transfusions

[35]. In a multiethnic country like Brazil, characterized by marked regional differences throughout the territory, it is important to study different populations regarding the distribution of platelet antigens to access regional differences and to detect the need for the establishment of panels composed of regional blood donors.

In countries with multi-ethnic and admixed populations, the risk of HPA alloimmunization may be higher than that observed in more homogeneous populations [36, 37]. This may occur due to a greater mismatch probability caused by the chance of ethnic differences between donors/patient (increasing the risk of alloimmunization by transfusion) and mothers/fathers (increasing the risk of NAIT). This information indicates the need of further studies on the incidence of NAIT and other alloimmune thrombocytopenic disorders in populations of South America, where these conditions seems to be underestimated [38].

This is the first report on the HPA allele frequencies in Belo Horizonte and represents the initial step toward the development of a genotyped platelet donor registry in order to treat HPA-alloimmunized patients in this region. We found a frequency of 0.1425 for the allele HPA-1b in Belo Horizonte (table 3). Considering that the blood donors represent the general population reasonably well and assuming that the population is in Hardy-Weinberg equilibrium, the frequency of HPA-1a-negative individuals is estimated to be 2.03%. This relatively high frequency of HPA-1a-negative individuals highlight the risk of NAIT in this region, since antibodies against HPA-1a account for more than 80% of cases of this clinical condition in Africans and Europeans [4]. At the same time, HPA-1a/5b-negative platelet donors were identified (2%) and are poten-

tial compatible donors of platelets for the treatment of NAIT, since transfusion with platelet concentrates with this haplotype are effective in the treatment of over 95% of cases of NAIT caused by maternal HPA antibodies [39, 40].

Considering that several donor characteristics, in addition to HPA frequencies, are relevant for the establishment of panels effective in meeting the transfusion needs [41] and that the incidence of alloimmune thrombocytopenic disorders in our populations is unknown, future studies to estimate the number of genotyped donors necessary to establish an appropriate platelet donor panel in Belo Horizonte are important.

In conclusion, we confirmed the heterogeneity of HPA antigens in Brazilian populations, reinforcing the importance of HPA panels composed of regional blood donors, or a national panel that contemplates the specificities of the different regions of the country, in order to provide support in platelet transfusions and minimize the risks associated with HPA alloimmunization.

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Disclosure Statement

The authors report no declarations of interest.

References

- Curtis BR, McFarland JG: Human platelet antigens (2013). *Vox Sang* 2014;106:93-102.
- Mueller-Eckhardt C, Kiefel V, Santos S: Review and update of platelet alloantigen systems. *Transfus Med Rev* 1990;4:98-109.
- Pappalardo PA, Secord AR, Quitevis P, Haimowitz MD, Goldfinger D: Platelet transfusion refractoriness associated with HPA-1a (PI(A1)) alloantibody without coexistent HLA antibodies successfully treated with antigen-negative platelet transfusions. *Transfusion* 2001;41:984-987.
- Davoren A, Curtis BR, Aster RH, McFarland JG: Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. *Transfusion* 2004;44:1220-1225.
- Conti F, Bertrand G, Dezan M, Costa T, Aravechia M, Mota M, Castilho L, Kaplan C, Kutner J: Molecular HPA genotyping by microarray in Brazilian blood donors. *Transfusion* 2014;54:405-411.
- Chen L, Liu Z, Liu T, Ma X, Rao M, Wang Y, Sun B, Yin W, Zhang J, Yan B, Li X, Wang Q, Zhang L, Wen J, Liu F, Wang P, Wei Y, Huang Y, Wu J, Guo Y, Kang Y, Song X, Liu X, Zhang G, Xie T, Chen Y, Zeng X: Neonatal alloimmune thrombocytopenia caused by anti-HPA antibodies in pregnant Chinese women: a study protocol for a multicentre, prospective cohort trial. *BMC Pregnancy and Childbirth* 2017;17:281.
- Ohto H, Miura S, Ariga H, Ishii T, Fujimori K, Morita S; Collaborative Study Group: The natural history of maternal immunization against foetal platelet alloantigens. *Transfus Med* 2004;14:399-408.
- Arinsburg SA, Shaz BH, Westhoff C, Cushing MM: Determination of human platelet antigen typing by molecular methods: importance in diagnosis and early treatment of neonatal alloimmune thrombocytopenia. *Am J Hematol* 2012;87:525-528.
- Kehdy FS, Gouveia MH, Machado M, Magalhães WC, Horimoto AR, Horta BL, Moreira RG, Leal TP, Scliar MO, Soares-Souza GB, Rodrigues-Soares F, Araújo GS, Zamudio R, Sant Anna HP, Santos HC, Duarte NE, Fiaccone RL, Figueiredo CA, Silva TM, Costa GN, Beleza S, Berg DE, Cabrera L, Debortoli G, Duarte D, Ghirotto S, Gilman RH, Gonçalves VF, Marrero AR, Muniz YC, Weissensteiner H, Yeager M, Rodrigues LC, Barreto ML, Lima-Costa MF, Pereira AC, Rodrigues MR, Tarazona-Santos E; Brazilian EPIGEN Project Consortium: Origin and dynamics of admixture in Brazilians and its effect on the pattern of deleterious mutations. *Proc Natl Acad Sci U S A* 2015;112:8696-8701.
- Castro V, Origa AF, Annichino-Bizzacchi JM, Soares M, Menezes RC, Gonçalves MS, Costa FF, Arruda VR: Frequencies of platelet-specific alloantigen systems 1-5 in three distinct ethnic groups in Brazil. *Eur J Immunogenet* 1999;26:355-360.
- Silvestre APA, Zacarias JMV, Guelsin GAS, Visentainer JEL, Sell AM: Genetic polymorphisms of human platelet antigens in Euro-African and Japanese descendants from Parana, Southern Brazil. *Platelets* 2017;28:607-610.
- The 1000 Genomes Project Consortium: A global reference for human genetic variation. *Nature* 2015;526:68-74.
- Halle L, Bigot A, Mullen-Imandy G, M'Bayo K, Jaeger G, Anani L, Martageix C, Bianchi F, Julien E, Kaplan C: HPA polymorphism in sub-Saharan African populations: Beninese, Cameroonians, Congolese, and Pygmies. *Tissue Antigens* 2005;65:295-298.
- De La Vega ECD, Nogués N, Fernández Montoya A, Chialina S, Blazaco PD, Theiller E, Raillon MA, Arancegui N, Solis E, Oyonarte S, Crespo Ferrer V, Campos Muñoz A, Muñoz-Díaz E: Human platelet-specific antigens frequencies in the Argentinean population. *Transfus Med* 2008;18:83-90.
- Chiba AK, Bordin JO, Kuwano ST, Figueiredo MS, Carvalho K, Vieira-Filho JPB, Kerbauy J: Platelet alloantigen frequencies in Amazon Indians and Brazilian blood donors. *Transfus Med* 2000;10:207-212.
- Feng ML, Liu DZ, Shen W, Wang JL, Guo ZH, Zhang X, Du KM, Qian KC, Zhao TM: Establishment of an HPA-1- to -16-typed platelet donor registry in China. *Transfus Med* 2006;16:369-374.
- Xu X, Zhu F, Ying Y, Tao S, Liu Y, Hong X, Yan L: Simultaneous genotyping of human platelet antigen-1 to 17w by polymerase chain reaction sequence-based typing. *Vox Sang* 2009;97:330-337.
- Halle L, Bach KH, Martageix C, Bianchi F, Lê T Kim T, Morel-Kopp MC, Soulier J, Tetaria C, Kaplan C: Eleven human platelet systems studied in the Vietnamese and Ma'ohis Polynesian populations. *Tissue Antigens* 2004;63:34-40.

- 19 Macher S, Schallmoser S, Ulrich S, Posch U, Wagner T, Lanzer G: Gene frequencies of human platelet antigens HPA-1, -2, -3 and -5 in Styria and establishment of a panel of HPA-typed platelet donors. *Transfus Med Hemother* 2004; 31(suppl 1):59 (abstract PO0703).
- 20 Korinkova P, Suttner J, Vytiskova J, Stehlikova M: Fetomaternal alloimmune thrombocytopenia – possibilities of diagnosis and exchange transfusion: *Ceska Gynekol* 1999;64:28–31.
- 21 Klüter H1, Fehlau K, Panzer S, Kirchner H, Bein G: Rapid typing for human platelet antigen systems-1, -2, -3 and -5 by PCR amplification with sequence-specific primers: *Vox Sang* 1996;71:121–125.
- 22 Mazzucco L, Santi R, Contino L: HPA-1/6 allelomorphic study in juvenile stroke: the possible role of HPA-2b and HPA 5b. 6th European Symposium on Platelet, Granulocyte and Red Cell Immunobiology (Abstract Book) Amsterdam. 2000, p 22.
- 23 Rozman P, Drabbel J, Schipper RF, Doxiadis I, Stein S, Claas FH: Genotyping for human platelet-specific antigens HPA-1, -2, -3, -4 and -5 in the Slovenian population reveals a slightly increased frequency of HPA-1b and HPA-2b as compared to other European populations. *Eur J Immunogenet* 1999;26:265–269.
- 24 Boehlen F, Bulla O, Michel M, Reber G, de Moerloose P: HPA-genotyping and antiplatelet antibodies in female blood donors. *Hematol J* 2003;4:441–444.
- 25 Portela CN, Schriefer A, Albuquerque SR, Perdomo RT, Parente AF, Weber SS: The human platelet alloantigen profile in blood donors from Amazonas, Brazil. *Transfus Med* 2016;26:448–456.
- 26 Merzoni J, Fagundes IS, Lunardi LW, Lindenau JD, Gil BC, Jobim M, Dias VG, Merzoni L, Sekine L, Onsten TG, Jobim LF: Human platelet antigen genotyping of platelet donors in southern Brazil. *Int J Immunogenet* 2015;42:329–335.
- 27 Bianchi JV, Azevedo MR, Jens E, Nukui Y, Chamone DA: Frequency of human platelet antigens in oncohematological patients with thrombocytopenia and the probability to platelet transfusions. *Rev Bras Hematol Hemoter* 2012;34:202–205.
- 28 Metsalu T, Vilo J: ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res* 2015; 43:W566–W570.
- 29 Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
- 30 Felsenstein J: PHYLIP – Phylogeny Inference Package (Version 3.2). *Cladistics* 1989;5:164–166.
- 31 Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, Kohlrausch F, Magno LA, Montenegro RC, Moraes MO, de Moraes ME, de Moraes MR, Ojopi EB, Perini JA, Racciopi C, Ribeiro-Dos-Santos AK, Rios-Santos F, Romano-Silva MA, Sortica VA, Suarez-Kurtz G: The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 2011;6:e17063.
- 32 Salzano FM: Molecular variability in Amerindians: widespread but uneven information. *An Acad Bras Cienc* 2002;74:223–263.
- 33 Covas DT, Biscaro TA, Nasciutti DC, Guerreiro JF, Santos SEB, Zago MA: Gene frequencies of the HPA-3 and HPA-5 platelet antigen alleles among the Amerindians. *Eur J Haematol* 2000;65:128–131.
- 34 da Silva MC, Zuccherato LW, Lucena FC, Soares-Souza GB, Vieira ZM, Pena SD, Martins ML, Tarazona-Santos E: Extensive admixture in Brazilian sickle cell patients: implications for the mapping of genetic modifiers. *Blood* 2011;118:4493–4495.
- 35 Verran J, Grey D, Bennett J, Lown JA, Erber WN: HPA-1, 3, 5 genotyping to establish a typed platelet donor panel. *Pathology* 2000;32:89–93.
- 36 Edinur HA, Dunn PP, Lea RA, Chambers GK: Human platelet antigens frequencies in Maori and Polynesian populations. *Transfus Med*. 2013;23:330–337.
- 37 Wan Syafawati WU, Norhalifah HK, Zefarina Z, Zafarina Z, Panneerchelvam S, Norazmi MN, Chambers GK, Edinur HA: Allele frequencies of human platelet antigens in Banjar, Bugis, Champa, Jawa and Kelantan Malays in Peninsular Malaysia. *Transfus Med* 2015;25: 326–332.
- 38 Castro V, Kroll H, Origa AF, Falconi MA, Marques SB, Marba ST, Passini R Jr, Annichino-Bizzacchi JM, Costa FF, Santoso S, Arruda VR: A prospective study on the prevalence and risk factors for neonatal thrombocytopenia and platelet alloimmunization among 9332 unselected Brazilian newborns. *Transfusion* 2007; 47:59–66.
- 39 Berry JE, Murphy CM, Smith GA, Ranasinghe E, Finberg R, Walton J, Brown J, Navarrete C, Metcalfe P, Ouwehand WH: Detection of Gov system antibodies by MAIPA reveals an immunogenicity similar to the HPA-5 alloantigens. *Br J Haematol* 2000;110:735–742.
- 40 Ouwehand WH, Smith G, Ranasinghe E: Management of severe alloimmune thrombocytopenia in the newborn. *Arch Dis Child Fetal Neonatal Ed* 2000;82:F173–175.
- 41 Ranasinghe E, Walton JD, Hurd CM, Saul L, Smith G, Campbell K, Ouwehand WH: Provision of platelet support for fetuses and neonates affected by severe fetomaternal alloimmune thrombocytopenia. *Br J Haematol* 2001;113:40–42.