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# Platelet alloantigen frequencies in Amazon Indians and Brazilian blood donors

A. K. Chiba,* J. O. Bordin,* S. T. Kuwano,* M. S. Figueiredo,* K. I. Carvalho,* J. P. B. Vieira-Filho† and J. Kerbauy* **Hematology and Transfusion Medicine Service and †Endocrinology Service, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, SP, Brazil*

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SUMMARY. The frequencies of human platelet-specific alloantigens (HPAs) vary between different ethnic groups, and genotyping using DNA techniques has been preferred over immunophenotyping methods for population studies. Using a polymerase chain reaction with allele-specific primers (PCR-ASP) method, we determined the allelic polymorphisms of five HPA systems among 174 unrelated individuals of two different Brazilian ethnic groups including Amazon Indians ($n = 95$) and blood donors ($n = 79$). Comparison of the calculated gene frequencies of the two alleles of HPA-1, -2, -3, -4 and -5 systems for Amazon Indians and Brazilian blood donors showed that gene frequencies obtained for the two alleles of HPA-1 ($P < 0{\cdot}001$), HPA-2 ($P = 0{\cdot}001$) and HPA-5 ($P < 0{\cdot}001$) were significantly different between the two groups of individuals. All natives tested carried the *HPA-2a* and the *HPA-5a* alleles, but the *HPA-1b* and *HPA-4b* alleles are absent from the Indian population. It was also observed that all blood donors carried the *HPA-1a*, *HPA-4a* and *HPA-5a* alleles. In conclusion, the present data indicate differences in the frequency of the HPA systems between Amazon Indians and Brazilian subjects who present a high rate of racial admixture. While the frequencies of the *HPA-1* and *HPA-5* genes seen in Amazon Indians are similar to those reported for Oriental populations, the frequencies of the HPAs alleles in Brazilian blood donors are comparable to those reported for populations in North America and Europe.

*Key words*: allelic polymorphisms, Amazon Indians, blood donors, human platelet antigens, PCR-ASP.

Human platelet-specific alloantigens (HPAs) are the target of platelet alloantibodies which can be formed during pregnancy or after the transfusion of blood components. The platelet alloantibodies can cause neonatal alloimmune thrombocytopenia and post-transfusion purpura (Kunicki & Beardsley, 1989; Mueller-Eckhardt *et al.*, 1989). Platelet-reactive alloantibodies have also been implicated in refractoriness to platelet transfusions, post-transplantation thrombocytopenia and passive alloimmune thrombocytopenia. The frequencies of HPAs vary between different populations (Santoso *et al.*, 1993; Kim *et al.*, 1995; Klüter *et al.*, 1996) and for this reason the investigation of the allelic polymorphisms of HPAs is important not only for anthropological and genetic reasons but also to better predict the risk for alloimmunization for HPAs among distinct populations.

Correspondence: Dr José Orlando Bordin, Hematology and Transfusion Medicine Service, Universidade Federal de São Paulo, Rua Botucatu, 740, São Paulo—SP-04023-902, Brazil. Tel.: (55 11) 574 0139; fax: (55 11) 571 8806; e-mail: jobordin@hemato.epm.br



Typically, immunophenotyping procedures have been used to perform studies on the HPAs; however, with the elucidation of the molecular basis of HPAs, genotyping using DNA techniques has been preferred for population studies (Kunicki & Newman, 1992). The frequencies of HPAs have been studied mainly in North American, European and Oriental populations (Santoso *et al.*, 1993; Holensteiner *et al.*, 1995; Kim *et al.*, 1995; Urwijitaroon *et al.*, 1995; Seo *et al.*, 1998). Employing a polymerase chain reaction (PCR) with allele-specific primers (PCR-ASP) method, we determined the frequency of allelic polymorphisms of HPAs among unrelated individuals from two different Brazilian ethnic groups, Amazon Indians and blood donors.

## MATERIALS AND METHODS

### *Blood samples*

Aliquots of EDTA-anticoagulated peripheral venous blood were collected from 79 unrelated healthy blood

donors in São Paulo City, Brazil, all of whom were of Brazilian origin. To ensure that these individuals represented a group with a high rate of racial admixture, they were selected according to an ancestry questionnaire, lip and hair thickness, skin and eye colour by two independent investigators. The blood donor population consisted of 47 (59·5%) men and 32 (40·5%) women.

Venous blood from 95 Amazon Indians (Xikrin-Kayapo Indians), who live in the south-east Brazilian Amazon basin and do not show racial admixture with other tribes or human races, was drawn into tubes containing heparin. Immediately after collection, the whole blood samples were frozen and shipped to our laboratory located at the Universidade Federal de São Paulo, São Paulo City, SP, Brazil. The native population consisted of 48 (50·5%) men and 47 (49·5%) women.

### *Genomic DNA*

DNA was extracted from leucocytes by phenol-cloroform, precipitated with ethanol and quantified by spectrophotometry (Sambrook *et al*., 1989).

### *Genotyping by PCR-ASP*

The genotypes of HPA-1, -2, -3, -4 and -5 were determined using the PCR-ASP method. In these studies we used three primers, two of which were allele specific with the 3′ base corresponding to the nucleotide that defines the polymorphism. The third primer in each system corresponds to the downstream consensus region. An HGH primer was used as internal amplification control in each reaction (Skogen *et al*., 1994; Cavanagh *et al*., 1997).

The PCR were performed using a Perkin-Elmer Cetus DNA Thermal Cycler in 50-$\mu$L reactions containing 250 ng genomic DNA, 2.5 U of *Taq* polymerase (Gibco BRL, Rockville, MD, USA), 10 mM Tris-HCl (pH = 8·3), 50 mM KCl, 0·01% gelatin, 1·5 mM $MgCl_2$ except for HPA-5 (3·0 mM $MgCl_2$), 200 $\mu$M of each dNTP, 0.5 $\mu$M for the HPA primers except for HPA-3 (1·0 $\mu$M), and HGH primers (0·1 $\mu$M). The mixture was overlaid with 100 $\mu$L of mineral oil.

After an initial denaturation step of 5 min at 94 °C, 30 cycles of PCR were performed under the following conditions: a denaturation step at 94 °C for 1 min; a 2-min annealing step at 62 °C for HPA-1a, -1b, -2a, -2b and -5b reactions; at 63 °C for HPA-3a; at 64 °C for HPA-3b; and at 60 °C for HPA-5b; and an extension step at 72 °C for 1 min. An additional 10 min at 72 °C was allowed at the end of each amplification. The samples were loaded onto a 1·6% agarose gel and stained with ethidium bromide. The gel was electrophoresed for 60 min at 80 V in TBE 1×, and visualized under UV illumination.

### *Statistical method*

The differences between gene frequencies of the two alleles of HPA-1, -2, -3, -4 and -5 detected in Amazon Indians and Brazilian blood donors were analysed by the $\chi^2$ or Fisher's exact test. The significance level was chosen to be 0·05.

## RESULTS

Examples of the results obtained by the PCR-ASP method are shown in Fig. 1. These results show that the designated primers specifically amplify the desired platelet HPA-1-related DNA, and that the two alloantigen-specific primers clearly distinguish between the two alleles. The 429-bp amplification product of the HGH control primer was present in all lanes, confirming that the DNA amplification had occurred. Similar PCR amplification patterns were obtained with primers related to the platelet-specific alloantigens HPA-2, -3, -4 and -5. For each HPA system, three samples from individuals with genotypes a/a, a/b and b/b are shown.

Genotype and allele frequencies of HPA-1, -2, -3, -4 and -5 systems for Amazon Indians and Brazilian blood donors are summarized in Table 1. The results show that the *HPA-1b* and *HPA-4b* alleles are absent from the population of Amazon Indians. On the other hand, it is shown that the *HPA-2a, HPA-4a* and the *HPA-5a* alleles are present in all individuals of the Amerindian group. In addition, the results show that the *HPA-1a*, *HPA-4a* and *HPA-5a* alleles are present in all tested Brazilian blood donors.

Comparison of gene frequencies of the two alleles of *HPA-1*, *-2*, *-3*, *-4* and *-5* genes for Amazon Indians and Brazilian blood donors is also shown in Table 1. The gene frequencies obtained for the two alleles of *HPA-1* ($P < 0{\cdot}001$), *HPA-2* ($P = 0{\cdot}001$) and *HPA-5* ($P < 0{\cdot}001$) in Amazon Indians were significantly different from those seen in the Brazilian blood donors.

## DISCUSSION

Investigation of the allelic polymorphisms of HPA is important not only for anthropological and genetic reasons but also to better predict the risk for alloimmunization for HPAs among distinct ethnic groups. PCR-ASP represents a rapid and technically simple method for detecting mutations and polymorphisms in population studies (Skogen *et al*., 1994; Cavanagh *et al*., 1997). This method uses primers with a base substitution at the 3′ position, based on the fact that the *Taq* polymerase does not extend efficiently when the 3′ end nucleotide is not perfectly complementary to the DNA template causing discrimination in allele

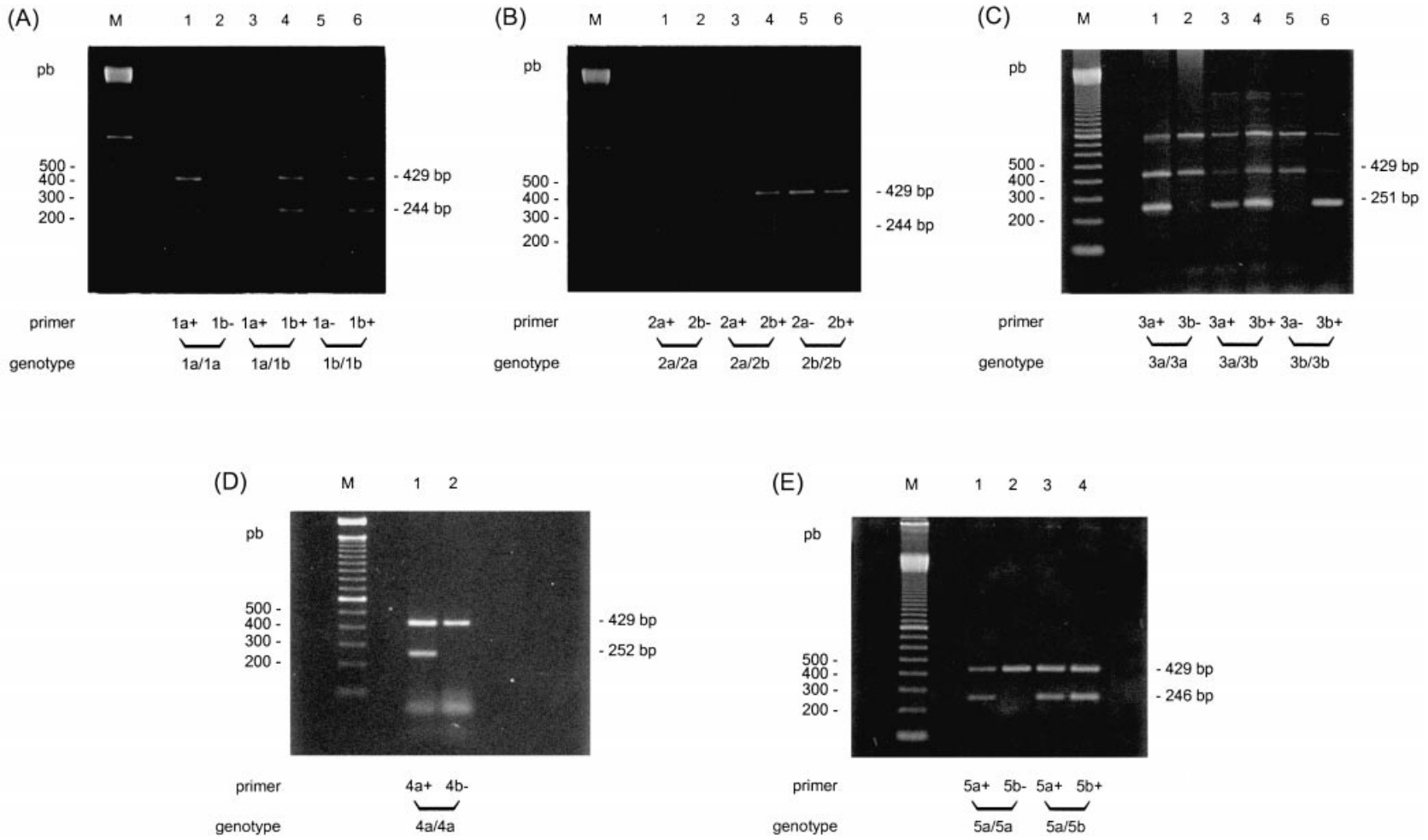


**Fig. 1.** Polymerase chain reaction with allele-specific primers (PCR-ASP) detection of HPA-1, -2, -3, -4 and -5 genotypes. Lanes 1 and 2 (panel A) represent an HPA-1a/a homozygous individual because a distinct band of 244 bp is present only in lane 1. The bands which represent an HPA-1a/b heterozygous individual are shown in lanes 3 and 4, while an example of an HPA-1b/b homozygous individual is shown in lanes 5 and 6. Panels B, C, D and E show the genotyping studies for the HPA-2, -3, -4 and -5, respectively. The HGH (control) PCR product (429 bp) is present in all reactions. These studies demonstrate that the designated primers specifically amplify the desired platelet alloantigen-related DNA, and that the two alloantigen-specific primers clearly distinguish between the two alleles.

**Table 1.** Genotype and gene frequencies of five HPAs in two distinct ethnic Brazilian groups

| | Genotype frequency | | Gene frequency | | |
|---|---|---|---|---|---|
| Genotype | Amazon Indians $n = 95$* | Blood donors $n = 79$† | Amazon Indians | Blood donors | *P*-value |
| HPA-1a/1a | 95 (100·0%) | 66 (83·54%) | 1·00 | 0·918 | |
| HPA-1a/1b | 0 | 13(16·46%) | | | < 0·001 |
| HPA-1b/1b | 0 | 0 | 0·0 | 0·082 | |
| HPA-2a/2a | 88 (92·63%) | 60 (75·95%) | 0·963 | 0·816 | |
| HPA-2a/2b | 7 (7·37%) | 16 (20·25%) | | | 0·001 |
| HPA-2b/2b | 0 | 3 (3·80%) | 0·037 | 0·139 | |
| HPA-3a/3a | 43 (48·31%) | 33 (42·30%) | 0·708 | 0·640 | |
| HPA-3a/3b | 40 (44·95%) | 34 (43·60%) | | | NS |
| HPA-3b/3b | 6 (6·74%) | 11 (14·10%) | 0·292 | 0·360 | |
| HPA-4a/4a | 83 (100·0%) | 79 (100·0%) | 1·00 | 1·00 | |
| HPA-4a/4b | 0 | 0 | | | NS |
| HPA-4b/4b | 0 | 0 | 0·0 | 0·0 | |
| HPA-5a/5a | 88 (92·63%) | 50 (64·90%) | 0·963 | 0·825 | |
| HPA-5a/5b | 7 (7·37%) | 27 (35·10%) | | | < 0·001 |
| HPA-5b/5b | 0 | 0 | 0·037 | 0·175 | |

*Genotyping was performed on DNA from 83 Amazon Indians for HPA-4. †Genotyping was performed on DNA from 78 and 77 unrelated blood donors for HPA-3 and HPA-5, respectively. NS = not significant.

amplification. Using a PCR-ASP method, we determined the frequency of HPA genes among unrelated individuals of two different ethnic populations including Amazon Indians and Brazilian blood donors. In each reaction, a pair of primers of the *HGH* gene was included as an internal positive control to monitor the general quality of the PCR and to ensure that the enzymatic process had worked properly. A negative reaction was defined by the presence of only the internal control represented by a 429-bp DNA fragment, while a positive reaction was defined by the presence of the respective DNA fragment along with the internal control fragment as indicated in Fig. 1.

Using the PCR-ASP method, we have identified several differences in the gene frequencies of the HPAs in Amazon Indians and Brazilian blood donors. First, the *HPA-1b* allele is not found in Amazon Indians. These results are quite similar to those recently reported by a genetic study performed with allele-specific restriction analysis in six tribes of Amerindians (Covas *et al.*, 1997), and with the very low frequency or absence of the *HPA-1b* allele described in several Asian populations (Santoso *et al.*, 1993; Kim *et al.*, 1995; Tanaka *et al.*, 1995; Urwijitaroon *et al.*, 1995) as shown in Table 2. The Brazilian blood donor population with a high rate of racial admixture showed a significant higher frequency of the *HPA-1b* allele compared to that seen in the native population. Nevertheless, the gene frequencies of the *HPA-1* alleles seen among Brazilian blood donors are not different from those reported for several American, Australian and European white populations (Simsek *et al.*, 1993; Holensteiner *et al.*, 1995; Chen *et al.*, 1997a, b) and for African Americans (Kim *et al.*, 1995) (Table 2).

The low frequency of the *HPA-2b* allele seen in Amazon Indians in this study is in concordance with those observed in individuals from different Amerindian tribes (Covas *et al.*, 1997). Although the Brazilian blood donor population showed a significant higher frequency of the *HPA-2b* allele compared with that seen in the native population, the gene frequencies obtained of the *HPA-2* alleles among Brazilians are not different from those reported for several American, Australian and European white populations (Simsek *et al.*, 1993; Holensteiner *et al.*, 1995; Chen *et al.*, 1997a, b) and for African Americans (Kim *et al.*, 1995) (Table 2).

No data on the HPA-3 and HPA-5 have thus far been reported for Amazon Indians. As for the HPA-1 and HPA-2 systems, the *HPA-5b* allele presented a considerably lower frequency in Amazon Indians than in blood

**Table 2.** Gene frequencies of HPA in different populations

| HPA Allele | German[1] | Dutch[2] | Japanese[3] | Korean[4] | African American[5] | Caucasian American[6] | Indonesian[7] | Australian[8] | Aboriginal Australian[9] | Amazon Indian[10] | Brazilian[11] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 0·820 | 0·846 | 0·998 | 0·988 | 0·920 | 0·890 | 0·991 | 0·838 | 0·992 | 1·000 | 0·918 |
| 1b | 0·180 | 0·154 | 0·002 | 0·012 | 0·080 | 0·110 | 0·009 | 0·162 | 0·008 | 0·000 | 0·082 |
| 2a | 0·920 | 0·934 | 0·898 | 0·926 | 0·820 | 0·920 | ND | ND | ND | 0·963 | 0·861 |
| 2b | 0·080 | 0·066 | 0·102 | 0·077 | 0·180 | 0·090 | ND | ND | ND | 0·037 | 0·139 |
| 3a | 0·630 | 0·555 | 0·594 | 0·555 | 0·630 | 0·670 | 0·461 | 0·648 | 0·937 | 0·708 | 0·640 |
| 3b | 0·370 | 0·445 | 0·406 | 0·445 | 0·370 | 0·330 | 0·539 | 0·352 | 0·063 | 0·292 | 0·360 |
| 4a | ND | 1·000 | 0·990 | 0·990 | 1·000 | 1·000 | 0·997 | ND | ND | 1·000 | 1·000 |
| 4b | ND | 0·000 | 0·010 | 0·010 | 0·000 | 0·000 | 0·003 | ND | ND | 0·000 | 0·000 |
| 5a | 0·900 | 0·902 | ND | 0·978 | 0·790 | 0·890 | 0·954 | 0·930 | 0·847 | 0·963 | 0·825 |
| 5b | 0·100 | 0·098 | ND | 0·022 | 0·210 | 0·110 | 0·046 | 0·070 | 0·153 | 0·037 | 0·175 |

[1]Chen *et al*., 1997b; [2]Simsek *et al*., 1993; [3]Tanaka *et al*., 1995, 1996; [4]Seo *et al*., 1998; [5]Kim *et al*., 1995; [6]Kim *et al*., 1995; [7]Santoso *et al*., 1993; [8]Chen *et al*., 1997a; [9]Chen *et al*., 1997a; [10]this study; [11]this study.

donors, while the *HPA-3b* allele did not. According to these results, the *HPA-3* allele frequencies detected in Amazon Indians are different from those reported by molecular studies for Australian aborigines and Indonesian people (Santoso *et al*., 1993; Simsek *et al*., 1993; Kim *et al*., 1995; Tanaka *et al*., 1995; Chen *et al*., 1997a, b), while the *HPA-5* allele frequencies for Amazon Indians are different from those reported for African American and Australian aborigines (Kim *et al*., 1995; Chen *et al*., 1997a, b). The gene frequencies obtained for the *HPA-5* alleles among Brazilian blood donors are also different from those reported for an Australian Caucasian population (Simsek *et al*., 1993; Holensteiner *et al*., 1995; Chen *et al*., 1997a, b) and for African Americans (Kim *et al*., 1995) (Table 2).

The diallelic HPA-4 system is polymorphic in Asian populations and most of the platelet-immunizations against HPA-4a or HPA-4b have been reported in Japan. Population studies in the United States and Europe have demonstrated that the frequency of the *HPA-4b* allele is extremely low in Caucasian populations and in North American Indians (Table 2). Although limited by a relatively small number of studied samples, in the present investigation, we found that the *HPA-4b* allele is virtually absent in Brazilian individuals and Amazon Indians. These results are also in accordance with platelet phenotyping studies, which have reported an HPA-4b low frequency of 0·9% (1 in 112) in Mapuches Indians from Chile (Inostroza *et al*., 1988).

In conclusion, the present data indicate differences in the frequency of the HPA systems between Amazon Indians and Brazilian subjects who present a high rate of racial admixture. The calculated frequencies of the *HPA-1* and *HPA-5* genes observed in Amazon Indians are similar to those reported for Oriental populations. Although these findings could reflect a common ancestral origin, since native Americans are believed to have migrated from Asia, the frequencies of the *HPA-2*, *HPA-3* and *HPA-4* genes seen in Amazon Indians are not exactly the same as those described for Orientals. Moreover, the present data show that the frequencies of the HPA alleles in Brazilian blood donors are comparable to those reported for populations in North America and Europe. Further biological and anthropological studies are essential to gain a better understaning of the allelic polymorphisms of HPAs among populations with distinct racial settings.

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