Frequencies of platelet-specific alloantigen systems 1–5 in three distinct ethnic groups in Brazil

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Summary

The human platelet antigen (HPA) systems are related to immune platelet disorders as well as to the development of occlusive vascular disease. Several distinct biallelic HPA systems are known, and a heterogeneous distribution of HPA alleles has been described among distinct ethnic groups. In this study we genotyped 320 carefully selected individuals from three distinct ethnic groups in Brazil (Caucasians, Blacks and Amazonian Indians) for the HPA-1, -2, -3, -4 and -5 systems. A similar prevalence for all HPA alleles was found in Brazilians of Caucasian and Black descent. These data contrast with those reported for similar ethnic groups in other countries. Among the Amazonian Indians, no b allele of the HPA-1, -4 and -5 systems was identified. The data presented here could be useful in the diagnosis of alloimmune platelet disease, in genetic counselling and in the development of screening programmes for HPA-related diseases.

Introduction

The human platelet alloantigen (HPA) systems are immunological epitopes resulting from polymorphism in the genes encoding membrane glycoproteins (Kunicki & Newman, 1992).

Alloimmunization to HPA systems is recognized clinically as a severe bleeding disease resulting from maternal sensitization to paternal alloantigens on foetal platelets, known as neonatal alloimmune thrombocytopenic purpura—NATP (Mueller-Eckhardt *et al*., 1989). Although distinct from red cell alloimmunization to the Rh system, the severe thrombocytopenia due to alloimmunization can result in a life-threatening disease in 50% of cases of NATP during the first pregnancy. Neonatal thrombocytopenia is not a rare event and has been described in 0.9% of an unselected cohort of neonates, and immune thrombocytopenia can be detected in 0.3% of live births (Dreyfus *et al*., 1997). However, antenatal screening to detect foetuses with a risk of NATP is generally not performed when there is no previous family history of the condition.

Several distinct biallelic HPA systems are known, and a heterogeneous distribution of HPA alleles has been described in distinct ethnic groups (Kim *et al*., 1995; Noris *et al*., 1995; Tanaka *et al*., 1996; Santoso & Kiefel, 1998). In Caucasians with NATP, antibody to HPA-1 or HPA-5 systems has been detected in 80 and 19% of cases, respectively (Mueller-Eckhardt *et al*., 1989). However, in African-American and Asian populations screening for HPA-1 has not been recommended due to the low prevalence of the HPA-1b allele in this group (Kim *et al*., 1995). In fact, in the Japanese population, the HPA-4 system is the most commonly involved in the cases of NATP, while anti-HPA-1 has never been described (Tanaka *et al*., 1996).

Alloimmune thrombocytopenia related to HPA systems is also observed after platelet transfusion in two distinct situations: the rare post-transfusion purpura (PTP), and in multitransfused patient who are refractory to platelet transfusion therapy (Friedman *et al*., 1996; Mueller-Eckhardt, 1986).

Moreover, HPA systems are expressed by integrins in vascular endothelial cells and may serve as minor histocompatibility antigens in organ transplantation (Kekomäki *et al*., 1997). The problem of platelet antigen mismatching in blood transfusion as well as in organ transplantation could be clinically important (Friedman *et al*., 1996; Sirén *et al*., 1998). The HPA systems were also recently related to non-immune diseases such as the increased risk for coronary and cerebral arterial occlusive diseases among those individuals carrying the HPA-1b or the HPA-2b alleles (Weiss *et al*., 1996; Gonzalez-Conejero *et al*., 1998). Further studies, on patients with occlusive vascular disease from distinct ethnic groups, have failed to confirm these results (Odawara *et al*., 1996; Reuner *et al*., 1997; Ridker *et al*., 1997; Wagner *et al*., 1998). The ethnic origin of the Brazilian population is highly heterogeneous. It is composed of immigrants from Europe, Africa and Asia, as well as Amerindian

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groups, which results in a complex race admixture, and differs from previously studied populations. Thus, an increasing interest in determining the prevalence of HPA alleles in the general population led us to study a sample of the three major racial groups which comprise the Brazilian population.

Materials and methods

Ethnic groups

The population studied was from three distinct regions of Brazil and represented samples from the three major ethnic groups which make up the population of this country. The samples were collected by the authors after an interview with the subjects from the groups of both Caucasian and African origin to verify the absence of admixture in the last three generations.

The first group consisted of 100 non-related individuals of Caucasian descent (56 male, 44 female) with an average age of 34.2 years (range: 19–62 years) recruited by the author from the students, physicians, and hospital staff of the State University of Campinas, State of São Paulo, south-eastern Brazil. Their ancestors were usually from Italy, Spain, Portugal and Germany.

The second group consisted of 150 (93 males, 57 females) non-related Brazilian Blacks with an average age of 33 years (range: 12–64 years). The samples were collected randomly by M.S.G. from students, laboratory staff, and physicians at the Federal University of Bahia, State of Bahia, north-eastern Brazil, where the large majority of the population is of African descent derived from the slave trade (Curtin, 1969).

The third group was composed of 70 Amazonian Indians (37 males, 33 females) with an average age of 31.8 years (range: 11–70 years) from the Tupi tribe known as the Parakanã. Random samples were collected in two villages (Paranatinga and Maroxewara), which are approximately 50 km apart, in Oriental Amazonia by two of the authors (M.S. and R.C.M.) during a campaign to control viral hepatitis.

Method

Genomic DNA was isolated from peripheral blood cells using a standard method (Millar *et al*., 1988). Genotyping was done using PCR–RFLP or PCR–SSP (for HPA-4). The polymerase chain reaction (PCR) was performed with the primers described for the HPA-1 (Jin *et al*., 1993), HPA-2 (Unkelbach *et al*., 1995), HPA-3 (Simsek *et al*., 1993), HPA-4 (Skogen *et al*., 1994) and HPA-5 systems (Kalb *et al*., 1994).

Briefly, 500 ng of genomic DNA was amplified by PCR in 30 μL of a reaction mixture containing 10 mM Tris-HCl (pH 8.5), 1.5 mM $MgCl_2$, 50 mM KCl, 1.0 mM each of dATP, dGTP, dTTP and dCTP, 400 ng of each primer and 2 U of *Taq* DNA polymerase. The reactions were performed under the following conditions: for the HPA-1, 3 and 5 systems: 5 min at 94 °C followed by 35 cycles consisting of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min; for the HPA-2 system, similar conditions were used, except for annealing at 57 °C for 1 min. The PCR products were incubated overnight at 37 °C with endonucleases as follows: HPA-1 (*Nci*I or *Msp*I); HPA-2 (*BsaH*I); HPA-3 (*Fok*I); HPA-5 (*Mnl*I). The digestion products were separated by eletrophoresis in 2% agarose gels followed by staining with ethidium bromide (Fig. 1).

Genotyping for the HPA-4 system was performed using allele-specific primers for alleles HPA-4a and HPA-4b, described by Skogen *et al*. (1994). In each reaction, we included a pair of primers for the amplification of a fragment of the methylenetetrahydrofolate reductase gene (Froost *et al*., 1995) as an internal control for the presence of DNA and the PCR reaction.

Results

The results obtained in this study are shown in Table 1. The genotype frequencies for all the HPA systems studied among Brazilian Caucasians and Blacks were a good fit to Hardy–Weinberg equilibrium (results not shown).

The prevalence of the HPA-1b allele among Brazilian Caucasians (0.075) was lower than that reported for European and U.S. populations (average: 0.152; $P = 0.05$, $\chi^2 = 3.9$) (Holensteiner *et al*., 1995; Kekomäki *et al*., 1995; Kim *et al*., 1995; Steffensen *et al*., 1996) and similar to the prevalence among Brazilians (0.097) and North Americans (0.080) of African descent (Kim *et al*., 1995).

The prevalence of the HPA-2b allele was found to be higher among Caucasians from Brazil than among those from Europe or the U.S. (average: 0.085; $P = 0.01$, $\chi^2 = 5.7$) (Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Kim *et al*., 1995; Steffensen *et al*., 1996), and was similar to that reported among populations of African descent (0.190; $P = 0.89$) (Kim *et al*., 1995).

Analysis of allele frequencies of the HPA-3 and HPA-4 systems revealed no statistically significant difference between Caucasians from Brazil and those from other populations (Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Kim *et al*., 1995; Steffensen *et al*., 1996), or between Brazilian Blacks and African-Americans (Kim *et al*., 1995).

The prevalence of HPA-5 alleles was similar in the Brazilian and other Caucasian populations (Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Kim *et al*., 1995; Steffensen *et al*., 1996). In Brazilians of African descent, a lower prevalence of HPA-5b was detected when compared to that described in Americans of African descent (0.124 vs. 0.210, respectively) (Kim *et al*., 1995); however, this difference failed to reach statistical significance ($P = 0.07$).

Among the Parakanã no b allele of the HPA-1 system was identified, which is in accordance with previous published data on six other distinct groups of native Amazonian Indians (Covas *et al*., 1997). However, the HPA-2b allele showed a higher prevalence among Parakanãs (0.179) compared to that found in the other groups of Amazonian Indians ($P = 0.001$, $\chi^2 = 10.16$) (Covas *et al*., 1997), for whom no data concerning the

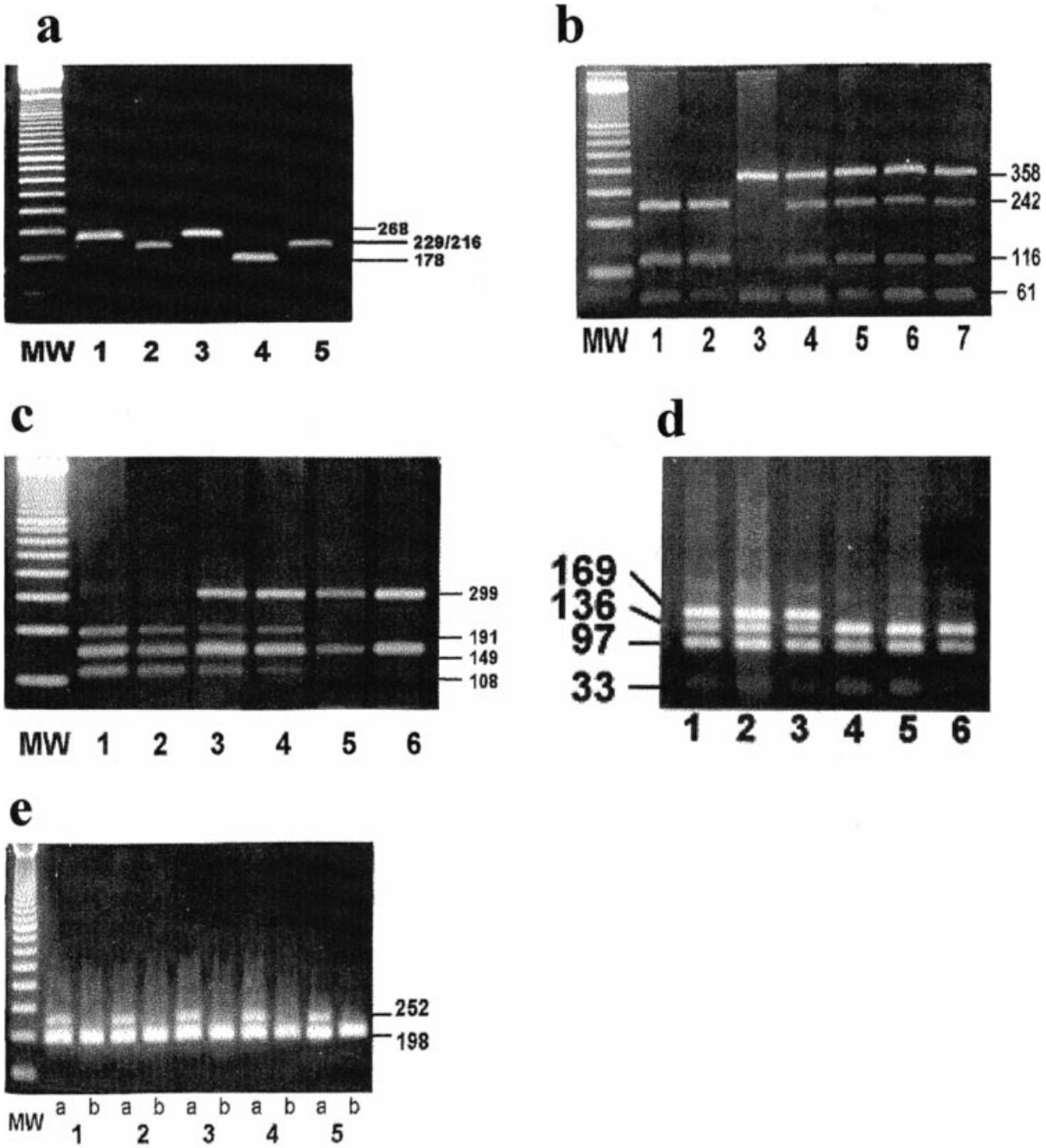

Figure 1. Ethidium bromide-stained 2% agarose gels showing PCR products corresponding to RFLP analysis for the HPA-1, -2, -3 and -5 systems and SSP analysis for the HPA-4 system. (a) HPA-1 system, NciI endonuclease: allele a = fragments of 268 bp; allele b = fragments of 216 and 52 bp. Homozygous for allele a — lanes 1 and 3; homozygous for allele b — lane 2. MW = 100-bp ladder molecular weight marker. HPA-1 system, MspI endonuclease: allele a = fragments of 39 (not seen) and 229 bp; allele b = fragments of 39, 51 (not seen) and 178 bp. Homozygous for allele a — lane 5; homozygous for allele b — lane 4. (b) HPA-2 system (BsaHI endonuclease): allele a = fragments of 61, 116 and 242 bp; allele b = fragments of 61 and 358 bp. Homozygous for allele a — lanes 1 and 2; homozygous for allele b — lane 3; heterozygous — lanes 4–7. MW = 100-bp ladder molecular weight marker. (c) HPA-3 system (FokI endonuclease): allele a = fragments of 108, 149 and 191 bp; allele b = fragments of 149 and 299 bp. Homozygous for allele a — lanes 1 and 2; homozygous for allele b — lanes 5 and 6; heterozygous — lanes 3 and 4. MW = 100-bp ladder molecular weight marker. (d) HPA-5 system (MnlI endonucleasis): allele a = fragments of 33 (not seen), 97 and 136 bp; allele b = fragments of 97 and 169 bp. Homozygous for allele a — lanes 4–6; heterozygous — lanes 1–3. (e) HPA-4 system (PCR–SSP): presence of the allele = fragments of 198 bp (PCR control — MTHF-R gene) and 252 bp; absence of the allele = only the fragment of 198 bp (PCR control — MTHF-R gene). a = lanes with allele a specific-primer; b = lanes with allele b specific-primer. All individuals (1–5) are homozygous for allele *a*. MW = 100-bp ladder molecular weight marker.

Table 1. Genotype and gene frequencies of HPA-1–5 systems in Amerindians, Brazilian Caucasians and Brazilian Blacks

	Caucasians (n = 100)					Blacks (n = 150)					Parakanã Indians (n = 70)				
	Genotype (%)			Gene frequency		Genotype (%)			Gene frequency		Genotype (%)			Gene frequency	
HPA	aa	ab	bb	a	b	aa	ab	bb	a	b	aa	ab	bb	a	b
1	86	13	1	0.925	0.075	82.6	15.4	2	0.903	0.097	100	—	—	1	0
2	73	24	3	0.850	0.150	65.4	31.3	3.3	0.810	0.190	71.4	21.4	7.2	0.821	0.179
3	37	46	17	0.600	0.400	45.4	42.6	12	0.666	0.334	58.5	34.3	7.2	0.757	0.243
4	100	0	0	1	0	100	0	0	1	0	100	0	0	1	0
5	85	14	1	0.920	0.080	76	23.3	0.7	0.876	0.124	100	—	—	1	0

HPA-3, -4 or -5 systems are currently available, to our knowledge. No b alleles of the HPA-4 and -5 systems were identified among the Parakanãs.

As the South Amerindians probably descend from Oriental populations, we decided to compare our data to those reported for Koreans, Chinese Indonesians and Japanese (Santoso *et al*., 1993; Tanaka *et al*., 1995; Chang *et al*., 1998; Seo *et al*., 1998). A similar prevalence of HPA-1a and HPA-5a alleles (allele frequency > 0.99) was found among these groups, except for HPA-5a in Indonesians (allele frequency: 0.954; not statistically significant). In contrast, the prevalence of the HPA-2, -3 and -4 system alleles among the Parakanã differed from those in Oriental populations (Santoso *et al*., 1993; Tanaka *et al*., 1995; Chang *et al*., 1998; Seo *et al*., 1998). The HPA-2b allele was more frequent among the Parakanãs (0.179) than in Koreans (0.077; $P = 0.009$, $\chi^2 = 6.84$) (Seo *et al*., 1998) or Chinese (0.025; $P = 0.0001$, $\chi^2 = 14.06$) (Chang *et al*., 1998). On the other hand, the prevalence of HPA-3b alleles was lower among the Parakanãs (0.243) than in Koreans (0.445; $P = 0.003$, $\chi^2 = 8.89$) (Seo *et al*., 1998), Chinese (0.475; $P = 0.001$, $\chi^2 = 9.81$) (Chang *et al*., 1998), Indonesians (0.539; $P = 0.00002$, $\chi^2 = 17.80$) (Santoso *et al*., 1993) or Japanese (0.406; $P = 0.03$, $\chi^2 = 4.58$) (Tanaka *et al*., 1995). No HPA-4b allele was identified among Parakanãs, as for Chinese (Chang *et al*., 1998), but contrasting with data obtained for Koreans (0.010) (Seo *et al*., 1998), Indonesians (0.003) (Santoso *et al*., 1993) and Japanese (0.010) (Tanaka *et al*., 1995).

Discussion

Data obtained in this study on selected groups of Caucasian and African descent in Brazil showed a distinct pattern from that reported for other countries (Mercier *et al*., 1994; Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Kim *et al*., 1995; Steffensen *et al*., 1996).

The prevalence of HPA-1b and HPA-2b alleles differed between Brazilian and European Caucasians (Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Steffensen *et al*., 1996), but was similar in Brazilians and North Americans of African descent (Kim *et al*., 1995). On the other hand, the prevalence of the HPA-3, HPA-4 and HPA-5 alleles was similar among Caucasians from Brazil, Europe (Holensteiner *et al*., 1995; Kekömaki *et al*., 1995; Steffensen *et al*., 1996) and the USA (Kim *et al*., 1995).

Among Brazilians of African descent, the prevalence of HPA-5b was 2-fold lower than that reported for African-Americans (Kim *et al*., 1995), but similar to that for Brazilian Caucasians. It is interesting to note that the origins of the Brazilian black population are mainly in Angola, Congo and Mozambique, unlike the African-Americans in which the majority of the studies on the HPA system have been carried out (Curtin, 1969; Kim *et al*., 1995). These data show a heterogeneous distribution of HPA alleles among groups of African as well as Caucasian descent from different countries. A possible reason for the equal prevalence of the HPA alleles among Brazilians of Caucasian and African descent may lie in a potential admixture of races in the past. However, when the population samples used in this study were analysed to determine the prevalence of the most frequent inherited risk factors for venous thrombosis, such as factor V Leiden and the prothrombin gene variant, we were able to show a clear difference between groups of Caucasian and African descent from Brazil. In fact, factor V Leiden and the prothrombin gene variant were found in 5 and 2% of Caucasians, respectively, and were rare (less than 1%) or absent among those of African descent (Arruda *et al*., 1995, 1997). Moreover, we have also determined the prevalence of the mutation in the methylenetetrahydrofolate reductase gene, related to neural tube defect (Froost *et al*., 1995), which was found in 10% of Caucasians from Brazil, and 1.5% of Brazilians of African descent (Arruda *et al*., 1998). These results are in accordance with those described previously both in Caucasians and in Africans (Motulsky, 1996; De Stefano *et al*., 1998; Rosendaal *et al*., 1998). Thus it is unlikely that racial admixture could account for the results described in this study. Further studies on the prevalence of HPA systems in larger populations where racial admixture is common would be of interest for comparison with our data.

South American Indians are probably related to Oriental populations and represent the descendants of migrating peoples originating in north-east Asia (Cavalli-Sforza *et al*., 1988). The prevalence of the HPA-1 and HPA-5 alleles was similar among the Amazonian Indians in this study and six distinct Amerindian groups

from the Brazilian Amazon and Chile, as well as Oriental populations, in other studies (Inostroza *et al*., 1988; Santoso *et al*., 1993; Tanaka *et al*., 1995.; Covas *et al*., 1997; Chang *et al*., 1998; Seo *et al*., 1998). However, the Parakanã differed from other native populations and Oriental populations when HPA-2, -3 and -4 were analysed. The Parakanã Indians represent a population almost unaffected by neo-Brazilians, with whom they only recently established regular contact. No clear evidence of European or African human leukocyte antigen or blood groups was found in members of this tribe born before 1974 (Black *et al*., 1980). They present distinct and typically Amerindian gene frequencies for the HLA system and some blood groups, such as AB0, Duffy and Diego. The lower degree of polymorphism in the Parakanã and in the other Amerindian groups compared to Blacks and Caucasians, as well as the differences in allele frequencies among the several Amerindian groups, are likely to result from random genetic drift (Black *et al*., 1980; Bailliet *et al*., 1994; Santos *et al*., 1996). In conclusion, the prevalence of HPA-1, -2, -3, -4 and -5 alleles among Brazilians of Caucasian and African descent was similar. The data presented here would be useful in diagnosis and genetic counselling and in the development of screening programmes for couples at risk of having babies with NATP. In addition, the small difference of HPA allele prevalence between Caucasian and Black Brazilians will not be of major clinical significance in platelet transfusion or organ transplantation. However, when individuals of Amerindian descent are considered for these procedures, these data suggest that compatibility for the HPA-1 and -5 systems should be carefully evaluated.

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