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Genetic Variability of Platelet Glycoprotein Ibα Gene

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Platelet membrane glycoprotein (GP) Ibα is a critical component of platelet adhesion complex to subendothelium structures following tissue injury or pathological surfaces, such as atherosclerotic plaques. Polymorphisms of the GPIbα gene have been associated with a high risk for occlusive vascular disease, and its distribution varies considerably among distinct populations. These polymorphisms comprise the human platelet antigen (HPA)-2 system, the –5C/T dimorphism of the Kozak sequence, and the variable number of tandem 39-bp repeats (VNTR). Here we report the prevalence of the GPIbα gene polymorphisms among Brazilians, a highly ethnically diverse population. We analyzed 492 subjects of European, African, or Indigenous origin. It was possible to determine ten distinct haplotypes. The most common (~40%) haplotype was the Kozak-TT/HPA-2aa/VNTR-CC for both Caucasian and African descent. However, among Indigenous, Kozak-TT/HPA-2aa/VNTR-CC and Kozak-TC/HPA-2aa/VNTR-CC were equally present. Although a strong linkage disequilibrium between VNTR and HPA-2 polymorphism had also been observed, here we determined incomplete linkage disequilibrium in 10% of subjects from all ethnic groups. VNTR-E, a rare variant lacking the 39-bp repeat, was identified in two unrelated subjects, and functional platelet studies revealed no abnormalities. The VNTR-A allele, the largest variant containing four copies of the repeats, was not identified in this population. However, homozygosity for the VNTR-A allele (Kozak-TT/HPA-2aa/VNTR-AA) was determined in two distinct species of nonhuman primates. These results suggest a greater complex evolutionary mechanism in the macroglycoprotein region of the GPIbα gene and may be useful in the design of gene–disease association studies for vascular disease. Am. J. Hematol. 77:107–116, 2004. © 2004 Wiley-Liss, Inc.

Key words: glycoprotein Ib alpha; polymorphism; platelet; genetic diversity

INTRODUCTION

Platelets have a central role in the blood coagulation by adhesion to exposed vascular subendothelium following vascular injury. Platelet adhesion is mediated by glycoprotein (GP) Ibα-Ibβ-IX-V complex, in which the GPIbα subunit contains binding sites for von Willebrand factor, thrombin, and actin-binding proteins [1]. Loss of function mutations in the human GPIbα gene is the most common molecular defect associated with the bleeding disorders known as Bernard-Soulier syndrome [2]. Disruption of the murine GPIbα gene recapitulates closely the human disease [3]. Platelets also participate in atherogenesis and subsequent formation of occlusive thrombi is dependent on platelet adhesion to the atherosclerotic plaque rupture [4].

Polymorphisms on the GP Ib-IX-V complex have been characterized. These polymorphisms are mostly restricted to the GPIbα subunit and can alter platelet

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antigenicity, regulate glycoprotein expression levels [5], and modulate its functional activity [6]. Thus, there is considerable interest in determining clinical implications of these platelet polymorphisms.

While two polymorphisms of the GPIbα gene were identified to affect the structure of the protein, another was found to influence the level of the receptor expression. Moroi et al. [7] described the existence of four size variants of GPIbα, which were characterized by Lopez et al. [8] as a variable number of tandem repeats (VNTR) of 39 bp that result in changes of 13 amino acid sequences extending from serine at position 399 to threonine 411. Ishida et al. [9] confirmed the presence of four alleles, named VNTR-A to VNTR-D, ranging from four repeats to one repeat, respectively. Recently, a rare variant characterized by the absence of a 13 amino acid repeat sequence has been reported, and it was named allele E [10]. Kuijpers et al. [11] described the polymorphism threonine at position 145 to methionine within the leucine-rich motifs, the molecular basis of the human platelet antigen system 2 (HPA-2). A strong linkage disequilibrium between VNTR and HPA-2 polymorphisms has been described [12]. However, data from African-Americans and Caucasians from Spain showed that this linkage is not complete [13,14]. Finally, Kaski et al. described a dimorphism in the 5′ untranslated sequence of GPIbα mRNA that lies within the Kozak sequence [15]. The levels of GPIb complex increase directly with the dosage of the −5C allele on platelet plasma membrane, ranging from 33% to 66% for heterozygous or homozygous, respectively.

Although not extensively explored, recently a notion of the functional consequences of distinct GPIbα polymorphisms was reported [16]. Analysis of platelet of 233 subjects with various GPIbα genotypes demonstrated that carriers of the Kozak TT allele or VNTR-CD presented 20% higher platelet plug formation under high shear stress.

An association between GPIbα polymorphisms and the development of occlusive vascular disease has been suggested by some studies [17–21] but not confirmed by others [22–25]. Several factors may have influenced these conflicting results such as selection criteria for patients, the time of patient enrollment (survivals of MI vs. acute MI event vs. sudden death), population size, age, ethnic background, and other potentially relevant factors.

Race is an important risk factor for the development of vascular diseases [26–28]. Brazilians form one of the most heterogeneous populations in the world as a result of interracial marriage of peoples from Europe and Africa in addition to the native Indigenous. Here we determine the prevalence of GPIbα polymorphisms among representative subjects of the three main ethnic groups in Brazil. These results demonstrated that the most frequent haplotype is commonly shared by individuals of African and European descent and, to a lesser extend, the Indigenous population. Linkage analysis of VNTR alleles to HPA-2 was found to be incomplete for all ethnic groups. While the VNTR-A allele was not identified among Brazilian subjects, in nonhuman primates double copies of the A allele were common findings. These data revealed a heterogeneous evolutionary mechanism of the GPIbα gene and may have implications for studies on disease–genotype association.

MATERIALS AND METHODS

This study was approved by local Committee of Ethics in Research from all three centers: State University of Campinas School of Medicine, Federal University of Bahia, and Evandro Chagas Institute.

Ethnic Groups

We analyzed a total of 492 individuals from three distinct regions in Brazil. Blood samples were collected after an interview, verifying that no mixture of race was known for the last three generations. Race was defined on the basis of ethnic background and not on physical characteristics or skin color, which have been demonstrated to have a high likelihood of misclassification [29].

The first group consisted of 280 non-related individuals of Caucasian descent (143 male, 137 female; median age, 40.7 years; range, 18–68 years), recruited from blood donors, laboratory staff, students and physicians of the State University of Campinas, State of São Paulo, southeastern Brazil. Ancestors were primarily from Italy, Spain, Portugal, Austria, and Germany.

The second group consisted of 145 subjects (79 male, 66 female; median age, 42.1 years; range, 12–72 years) from non-related Brazilians of African descent. The samples were collected randomly from students, laboratory staff, and physicians at the Federal University of Bahia, State of Bahia, northeastern Brazil, by M.S.G. This population represents those individuals who were initially introduced in the country centuries ago during slave trade and whose ancestors are mainly from Angola, Congo, and Mozambique [30].

The third group was composed of 67 Amazonian Indians (33 male, 34 female; median age, 34.4 years; range, 13–70 years) from the Tupi tribe named Parakanã. They are from two separate villages in the Oriental Amazonia (northern Brazil), in which miscegenation with other racial groups have not been

demonstrated [31]. Blood samples were collected by researchers from the Evandro Chagas Institute.

Nonhuman Primates

Genomic DNA from two different species of nonhuman primates, *Gorilla gorilla* (NG05251) and *Pan troglodytes* or chimpanzee (NG06939), were obtained from NIA Aging Cell Culture Repository (Coriell Institute for Medical Research, Camden, NJ) and genotyped using the same primers for studies in human samples.

Genotyping for VNTR Polymorphism of the GPIbα Gene

Identification of the VNTR polymorphism of the GPIbα gene was performed by two distinct polymerase chain reaction (PCR) assays as previous described by Gonzalez-Conejero et al. [18]. The first pair of primers were located at nucleotides 3915–3938 and 4378–4400, and the second pair of primers were located in 4202–4223 and 4378–4400. The PCR products were separated by electrophoresis in a 3% agarose gel and visualized under UV light after staining with ethidium bromide.

Genotyping for HPA-2

The characterization of the HPA-2 alleles was performed as reported by Castro et al. [32]. Following digestion of the PCR products with *Bsa*HI in the presence of HPA-2a, three fragments of 61, 116, and 242 bp were obtained, whereas in the presence of HPA-2b allele, only two fragments (61 and 358 bp) were observed.

Genotyping for Kozak Sequence Polymorphism

Identification of the Kozak polymorphism in the GPIbα gene was performed by PCR using primers corresponding to nucleotides 2757–2780 and 3203–3224 as previous described [24]. Amplified products were digested with *Ava*II (MBI Fermentas, Vilnius, Lithuania). The genotype −5T is identified by the presence of fragments of 131, 161, and 175 bp, whereas for the allele −5C only two fragments of 131 and 336 bp were obtained.

Molecular and Functional Characterization of the VNTR-E Allele

The PCR product using primers corresponding to nucleotides 4202–4223 and 4378–4400 of the VNTR-E allele was directly cloned into pGEM-T vector (Promega Corporation, Madison, WI). Four positive clones were further sequenced using the ABI-Prism® 377 DNA sequencer (PE Applied Biosystems, Foster City, CA). Blood samples were collected for characterization of platelet morphology and functional assays from subjects carrying the VNTR-E allele. The Ivy bleeding time, ristocetin-induced platelet aggregation (RIPA) test, and platelet aggregation to various agonists were performed as described [33]. Platelet glycoprotein content was determined in platelet-rich plasma. Binding of fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibody against GPIbα (CD42b-PE), GPIX (CD42a-FITC), GPIIb (CD41-FITC), and GPIIIa (CD61-FITC) (Beckman-Coulter Immunotech, Marseille, France) was determined by the direct method. Negative controls consisted of non-immune mouse IgG1-PE and IgG2a-FITC (Beckman-Coulter Immunotech). The fluorescence intensity was analyzed by flow cytometry (FACScan, Becton Dickinson, San Jose, CA), and a fluorescence histogram was obtained.

Statistical Analysis

The statistical significance of the differences between groups were calculated by χ^2 or Fisher's exact tests for small samples using the Epi Info program [34]. Differences were considered statistical significant for P values ≤ 0.05. The χ^2 goodness-of-fit test was applied to determine whether the observed genotype frequencies were in agreement with the Hardy–Weinberg equilibrium.

RESULTS

Frequencies of Genotypes and Haplotypes of the VNTR Polymorphism of the GPIbα

PCR products using the pair of primers located at nucleotides 3915–3938 and 4378–4400, resulted in fragments of 563 bp (allele B), 524 bp (allele C), 485 bp (allele D), and 446 bp (allele E). A second PCR assay using a pair of primers located in 4202–4223 and 4378–4400 resulted in the identification of four visible fragments of 276, 237, 198, and 159 bp corresponding to the alleles B, C, D, and E, respectively (Fig. 1). There was complete agreement on the results obtained by independent PCR assays. The genotype frequencies of VNTR among those with Caucasian ($\chi^2_{(3)} = 0.52$; $0.90 < P < 0.95$) or African descent ($\chi^2_{(2)} = 1.91$; $0.30 < P < 0.50$) fit the Hardy–Weinberg equilibrium well. This was further confirmed by the analysis of the distribution of the Kozak alleles among those with Caucasian ($\chi^2_{(1)} = 2.91$; $0.05 < P < 0.1$) or African descent ($\chi^2_{(1)} = 0.163$; $0.5 < P < 0.7$). Together these data suggest that these individuals are representative of their own populations.

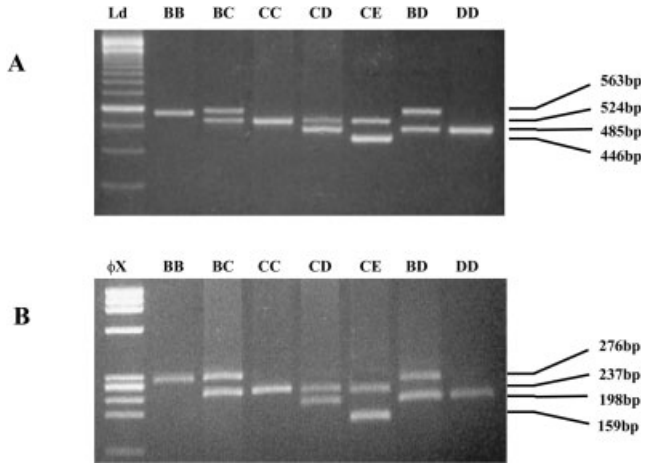


Fig. 1. VNTR alleles of GPIbα gene. Genotyping procedures from all subject samples were performed in two independent PCR assays, and the product is visualized in a 3% agarose gel stained with ethidium bromide. (A) Primers located at nucleotides 3915–3938 and 4378–4400 resulted in PCR products of 563, 524, 485, and 446 bp corresponding to VNTR alleles B, C, D, and E, respectively. Ld: molecular weight marker 100-bp ladder. (B) The same genomic DNA when amplified using primers located at positions 4202–4223 and 4378–4400 resulted in PCR fragments of 276, 237, 198, and 159 bp corresponding to VNTR alleles B, C, D, and E, respectively. φX: *Hae*III digest molecular weight marker.

TABLE I. Frequency of Genotype and Alleles of the Tandem Repeats Polymorphims of the GPIbα Gene Among Distinct Ethnic Groups in Brazil*

	Caucasians (n = 280)	Africans (n = 145)	Indigenous (n = 67)
Genotype (%)			
BB	5 (1.8%)	3 (2.1%)	2 (3%)
BC	46 (16.4%)	38 (26.2%)	12 (17.9%)
CC	187 (66.7%)	90 (62.1%)	53 (79.1%)
CD	31 (11.1%)	9 (6.2%)	0
DD	4 (1.4%)	3 (2.1%)	0
BD	5 (1.8%)	2 (1.3%)	0
CE	2 (0.8%)	0	0
Gene frequency			
B	0.109	0.158	0.119
C	0.809	0.783	0.881
D	0.079	0.059	0
E	0.003	0	0

*χ^2 test or Fisher's exact test for small samples was used to compare data among all groups. The frequency for the BC genotype was higher among Africans compared to Caucasians ($P < 0.02$; $\chi^2 = 5.75$), as well as for the B allele ($P < 0.04$; $\chi^2 = 4.28$). The frequency of the CC genotype or C allele was higher among Indigenous compared to Caucasians ($P < 0.05$; $\chi^2 = 3.84$, and $P < 0.05$; $\chi^2 = 3.8$, respectively). Comparison between Indigenous and Africans revealed higher frequency of the CC genotype ($P < 0.01$; $\chi^2 = 6.03$) and C haplotype ($P < 0.02$; $\chi^2 = 5.8$) among Indigenous.

However, the analysis of the GPIbα polymorphisms among Indigenous subjects revealed that the allelic distribution is not in equilibrium. Based on the HLA system and some blood groups, such as ABO, Duffy and Diego determined no miscegenation in these communities. However, several factors are likely to influence allelic distribution, such as high prevalence of polygamy, consanguineous marriage, and the bottleneck effect [31].

The distribution of the VNTR alleles among all subjects is shown in Table I. The prevalence of the genotype BC was higher among those of African descent than those of Caucasian descent (26.2% vs. 16.4%; $P < 0.02$; $\chi^2 = 5.75$). Similarly, the gene frequency of the B allele was higher for Africans when compared to Caucasians (0.158 vs. 0.109; $P < 0.04$; $\chi^2 = 4.28$). No other difference of the distribution of the VNTR alleles was determined among African and Caucasian subjects.

Among Indigenous subjects, no allele D was observed. When compared to the other racial groups, the genotype CC was higher among the Indigenous population (79.1%) than in those with Caucasian (66.7%) or African descent (62.1%). In addition, the gene frequency of the allele C was more prevalent among Indigenous subjects compared to those individuals with Caucasian or African descent ($P < 0.05$).

Frequencies of Genotypes and Haplotypes of the Kozak Dimorphism

Table II summarizes the frequencies of genotypes and haplotypes of the Kozak polymorphism among the three distinct ethnic groups. The gene frequencies of the allele −5C were similar between Brazilians of Caucasian and African descent (0.139 vs. 0.169,

TABLE II. Frequency of the −5C/T Kozak Dimorphism of the GPIbα Gene Among Distinct Ethnic Groups in Brazil*

	Caucasians (n = 280)	Africans (n = 145)	Indigenous (n = 67)
Genotype (%)			
TT	203 (72.5%)	99 (68.3%)	34 (50.7%)
TC	76 (27.1%)	43 (29.6%)	32 (47.8%)
CC	1 (0.4%)	3 (2.1%)	1 (1.5%)
Gene frequency			
T	0.861	0.831	0.746
C	0.139	0.169	0.254

*χ^2 test or Fisher's exact test for small samples was used to compare data among all groups. The TT genotype was higher among Caucasians or Africans compared to Indigenous ($P < 0.0006$; $\chi^2 = 11.78$, and $P < 0.01$; $\chi^2 = 6.0$, respectively). The TC genotype was higher among Indigenous compared to Caucasians ($P < 0.001$; $\chi^2 = 10.69$) or Africans ($P < 0.01$; $\chi^2 = 6.54$). No significant difference was found among Brazilian Caucasians and those of African descent.

TABLE III. GPIbα Gene Haplotype Among Brazilian Subjects

		Caucasians			Africans			Indigenous		
		Kozak dimorphism			Kozak dimorphism			Kozak dimorphism		
HPA-2	VNTR	TT	TC	CC	TT	TC	CC	TT	TC	CC
bb	BB	3	1		2			1	1	
bb	BC				1			2		
ab	BB	1			1					
ab	BC	35	10		23	10		7	3	
ab	BD	5				2				
ab	CC	8	4		8	4			3	
ab	CD	1			1					
aa	BC	1			2	1	1			
aa	CC	123	52	1	55	21	2	24	25	1
aa	CD	22	8		4	4				
aa	CE	1								
aa	DD	3	1		2	1				
Total	$n = 492$	203	76	1	99	43	3	34	32	1
Informative cases[a]	$n = 371$	162	54	1	75	22	3	27	26	1

[a]Informative cases refer to individuals presenting homozygosity for at least two distinct polymorphisms.

respectively; $P = 0.25$). Among Indigenous subjects, a higher prevalence of the −5C allele was determined when compared with Caucasians (0.254 vs. 0.139, respectively; $P < 0.001$; $\chi^2 = 10.45$) or Africans (0.254 vs. 0.169, respectively; $P < 0.04$; $\chi^2 = 4.17$).

Genetic Linkage of the GPIbα Polymorphisms

In a previous study, we reported the prevalence of HPA-1 to HPA-5 alleles in these ethnic groups [32]. Here we analyzed the genetic linkage of the HPA-2, VNTR, and Kozak polymorphisms of the GPIbα gene among subjects from all three ethnic groups (Table III). It was possible to determine ten distinct haplotypes. The most common was the association of Kozak-TT/HPA-2aa/VNTR-CC for Brazilians of Caucasian and African descent. However, among the Indigenous population, Kozak-TT/HPA-2aa/VNTR-CC and Kozak-TC/HPA-2aa/VNTR-CC were equally present.

Incomplete Linkage Disequilibrium of GPIbα Alleles

We observed an unexpected linkage between HPA-2b and VNTR-C in 32 of 340 informative (9.4%) cases. These informative individuals were carriers of VNTR-CC or HPA-2bb. The distribution according to race revealed that 14% of those with African descent, 10% of Indigenous, and 6% of Caucasians presented the association of HPA-2b/VNTR-C. In addition, the unexpected association of HPA-2a with VNTR-B allele was identified in 7 out of 361 (2%) of all informative cases (i.e., carriers of HPA-2aa or VNTR-BB alleles). However, the prevalence of the HPA-2a/VNTR-B was 5% among African descents, ~1% among Caucasians ($n = 2$) and absent among those of Indigenous descent. When we analyzed the relationship between Kozak polymorphism and the two other polymorphisms of the GPIbα, we observed that in the large majority, the Kozak-C allele was associated with the presence of VNTR-C or -D alleles and HPA-2a. However, two subjects (one of Caucasian and one of Indigenous descent) presented haplotype Kozak-TC/HPA-2bb/VNTR-BB, and one of African descent showed the Kozak-CC/HPA-2aa/VNTR-BC.

Carriers of the VNTR-CE Present Platelets With Normal Morphological and Functional Features

Interestingly, two non-related Caucasian subjects presented the rare variant E in addition to VNTR-C allele. In both cases, the sequence of the flanking region of the deletion revealed an additional neutral nucleotide change A to G at position 4301 that encodes proline 395 (Fig. 2). Blood samples from family members of one case were obtained for platelet phenotype and genotype assays (Fig. 3a,b). Ivy bleeding time, platelet counts, platelet volume, and morphology were all within the normal range. Platelet aggregation was normal in response to ADP (10 μM), collagen (10 μg/mL), epinephrine (10 μM), arachidonic acid (500 μg/mL), and to ristocetin at doses ranging from 0.7 to 1.2 μg/mL. The expression of GPIb/IX/V complex, GPIIb, and GPIIIa were analyzed by flow cytometry. As shown in Fig. 3b, the numbers of all constituents of the GPIb/IX/V complex were normal. The number of binding sites for anti-GPIIb and anti-GPIIIa were also within the normal range.

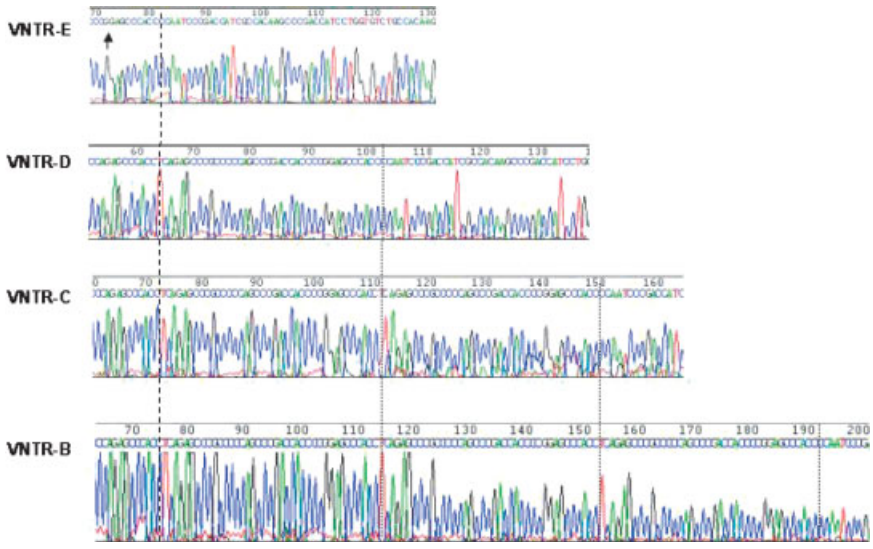


Fig. 2. Sequencing of the GPIα gene from human subjects. DNA sequence of the E allele is shown on the top, with no tandem repeat of the 39-bp sequence. The arrow demonstrated the neutral change A→G at position 4301. The VNTR alleles D, C, and B are shown from the top to the bottom. Tandem repeated sequences are flanked by dotted lines. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Homozygosity for the VNTR-A Allele Is Common Among Nonhuman Primates

We were not able to detect human subjects with VNTR-A allele. However, when we analyzed the genomic DNA obtained from nonhuman primates for the GPIbα polymorphisms, both animals carry the genotype Kozak-TT/HPA-2aa/VNTR-AA. The presence of the homozygosity of the VNTR-A allele was confirmed by the sequence of the PCR products corresponding to this region (Fig. 4).

DISCUSSION

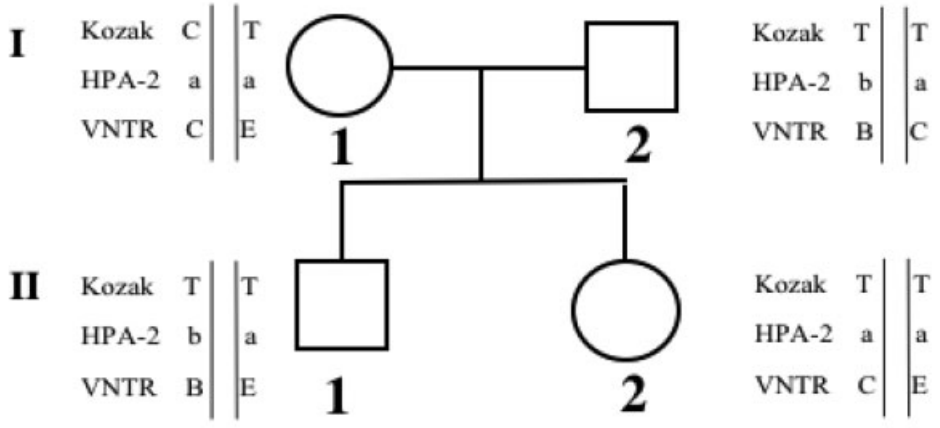
Brazil has one of the most ethnically heterogeneous populations in the world. The slaves, who originated from ethnically diverse African populations, admixed with each other as well as with people from European descent and the Indigenous of Brazil [29]. This makes the Brazilian gene pool highly heterogeneous.

Occlusive vascular disease is a prominent health problem in Brazil, especially among adults younger than 45 years [35–37]. To better understand the genetic basis of complex diseases such as atherosclerosis and occlusive vascular disorders, studies in both African and non-African populations are important to test the hypothesis that common disease often are caused by common susceptibility alleles (for review, see Tishkoff and Williams [38]). It is possible that Brazilians provide a good population to test this hypothesis, because subjects of distinct backgrounds living under similar environmental conditions may or may not have similar susceptibility to vascular disease. Thus, the identification of specific genes commonly distributed among these individuals could provide an opportunity to recognize gene pool-associated disease.

Several reasons lead us to exploit the distribution of GPIbα alleles among Brazilians, such as the central role of platelet adhesion in the atherosclerosis process and the unique characteristic of the GPIbα gene, which is encoded by a single exon [1,4,39]. All three characterized polymorphisms are located either in the promoter or in the coding region, which influence the amount of the receptor expression and/or its function [5,6,15,16]. Moreover, it is known that the distribution of the distinct alleles is heterogeneous among subjects of distinct ethnic backgrounds [8,12,14].

Overall, we determined that GPIbα VNTR polymorphisms present a highly heterogeneous distribution among Brazilians, markedly among those of African descent. Interestingly, the differences of the VNTR alleles among Brazilians of African or Caucasian descent were restricted to the genotype BC or B allele, which was higher among Africans than Caucasians (26% vs. 16%, respectively). Moreover, the prevalence of the VNTR alleles among African Brazilians also differs from that of African Americans [13]. Africa has a highly heterogeneous population, and these subjects have been understudied compared with non-African populations [38]. The heterogeneity among those of African descent can be explained, in part, by the distinct origin of the early population brought from Africa through slave trade. In Brazil, they originated mainly from Angola, Congo, and Mozambique, whereas African Americans descended from regions extending from Senegal to Western

A



B

Subjects	Platelet count (x 10^9 /L)	Mean Platelet Volume (fL)	Ivy Bleeding Time (mins)	RIPA * (mg/mL)	VNTR Genotype	Mean Fluorescence Intensity			
						Ibα (CD 42b)	IX (CD 42a)	IIb (CD 41)	IIIa (CD 61)
Normal Control	130 - 400	7.2 –11.1	2 – 9	0.7 - 1.2	CC (n=1)	85.06	708.12	65.87	1136.47
I.1	311	6.2	7.05	1.2	CE	113.42	677.38	72.15	753.88
I.2	389	6.6	5.87	1.1	BC	86.61	654.67	46.33	1340.95
II.1	419	6.1	5.97	0.8	BE	97.52	708.79	86.86	1147.09
II.2	143	7.3	4.22	1.2	CE	105.45	701.80	45.55	870.42

*RIPA: Ristocetin-induced platelet aggregation; minimal dose required to achieve more than 60% of platelet aggregation.

Fig. 3. Pedigree of the proband and family members with a rare VNTR-E variant. (A) The Kozak, HPA-2, and VNTR genotypes are represented for each family member. The arrow indicates the proband. (B) Platelet morphological, phenotypic, and functional characteristics; normal values represent data obtained from local laboratory controls (n = 50).

Nigeria and from Eastern Nigeria to Angola [30]. In contrast, no difference was determined regarding Kozak dimorphism when African Brazilians were compared to African Americans [5].

In contrast to those of African descent, comparison among Caucasians from Brazil with those from Europe or North America shows no difference in the distribution of the VNTR alleles or Kozak dimorphism [8,13,15].

Among Brazilians of Indigenous descent, the prevalence of VNTR allele C or Kozak −5C allele was higher compared to Brazilians of non-Indigenous descent. In addition, the prevalence of Kozak dimorphism in the Indigenous population was similar to that described among Oriental populations, e.g., Japanese or Korean [24]. However, the prevalence of VNTR alleles differs between Indigenous and the Oriental populations. Notably is the absence of the largest VNTR, the A allele, which is present in 8–15% of Oriental population [7,24,32]. The distinct prevalence of these alleles among Indigenous population in this study may be influenced by genetic drift or region-specific selection pressure and importantly, by the high rate of consanguineous marriage [40].

It is known that HPA-2 and VNTR alleles are in strong linkage disequilibrium. The largest VNTR alleles (alleles A and B) are associated with HPA-2b (the lesser frequent), while VNTR alleles C or D are associated with HPA-2a [8–12]. Overall, we report here that 10% of informative cases presented the allele HPA-2b/VNTR-C. The lowest prevalence was determined among Caucasians (6%), but this is the first demonstration of such association in this group. It is interesting to note that we identified 14% among those of African descent and 10% among the Indigenous population with HPA-2b/VNTR-C alleles,

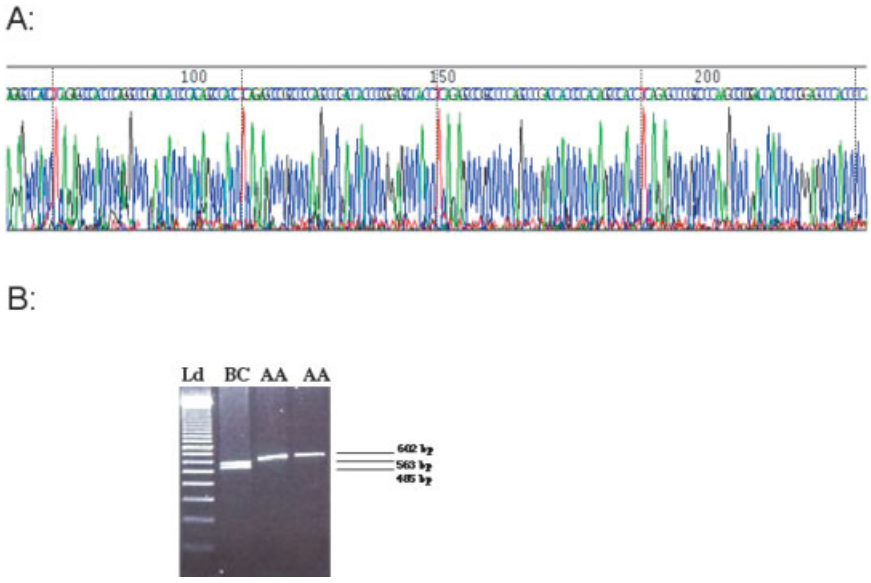


Fig. 4. Characterization of the GPIα gene from nonhuman primates. (A) Sequence of genomic DNA from gorilla demonstrated the A allele characterized by four tandem repeats. (B) PCR products using primers located at positions 3915–3938 and 4378–4400 resulted in a single large fragment of 602 bp (VNTR-A allele) using genomic DNA from gorilla or chimpanzee. Control DNA from a human subject previously characterized as a carrier of alleles BC is shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

which is 6-fold higher than the 2.2% among those of African descent as reported by Aramaki and Reiner [13]. The association of HPA-2a/VNTR-B was detected in ~6% of Caucasians from Spain or African Americans. It was reported that this is present in 5% of those of African descents and 1% among Caucasians [13,14]. Thus, we showed that the linkage disequilibrium in our population is not complete. Indeed, it is relatively common and affected all three ethnic groups.

We cannot rule out that admixture among individuals from distinct ethnic groups occurred in earlier generations, which could explaining some of the results observed in this study. However, this is an unlikely hypothesis because, using a similar criteria selection, we were able to demonstrate that mutations that were highly prevalent among Caucasians were not identified among African descents [41–43].

In addition, we have identified the rare VNTR null allele (VNTR-E) in two unrelated individuals of Caucasian descent. Together with a previous case reported by Muckian et al. [10], the variant VNTR-E has been reported only among Caucasians. Here the subjects were clinically asymptomatic regarding bleeding or thrombosis events. Platelet morphological, immunophenotype, and functional characterization of the affected subject and family members demonstrated no abnormality among carriers of the genotype VNTR-CE or VNTR-BE.

Initially, an evolutionary model of GPIbα haplotypes was proposed. In this model, the addition of extra copies to the initial VNTR allele of one copy (allele D) gave rise to larger VNTR [8,9,12]. Here we showed that GPIbα gene in two closest species of nonhuman primates, gorilla and chimpanzee, presented homozygosity for the VNTR-A alleles (four copies of 39 bp). We hypothesize that, instead of the addition of new copies with a series of tandem duplication, the evolutionary model could have been a result of loss of these copies. Thus, the VNTR-A should be considered the oldest allele and the subsequent deletions resulted in the smaller forms. Recently, Osawa et al. [44] reported a possible evolutionary origin of another VNTR present in the promoter region of the cystatin B gene, associated with human progressive myoclonus epilepsy. They have determined that, among nonhuman primates, this repeat segment has stable copies, in contrast to the variable forms observed in the same sequence in humans, suggesting that this VNTR arose after the humans and hominoids split. It was hypothesized that once the VNTR gained additional repeats units, as observed in early hominoid ancestors, the allele could became unstable and, during the human evolutionary process, lost the tandem repeats elements, resulting in the smallest variant as those detected in humans.

In summary, despite the differences in the distribution of several GPIbα alleles among subjects from distinct ethnic backgrounds, we were able to determine that the most common (~40%) haplotype was the Kozak-TT/HPA-2aa/VNTR-CC for individuals of Caucasian and African descent. However, among the Indigenous population, Kozak-TT/HPA-2aa/

VNTR-CC and Kozak-TC/HPA-2aa/VNTR-CC were equally present. Whether carriers of these alleles have increased susceptibility to vascular diseases in Brazil remains to be determined in large population studies.

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