Knops blood group haplotypes among distinct Brazilian populations

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BACKGROUND: The Knops blood group system consists of antigens encoded by exon 29 of complement receptor 1 (CR1) gene. To better elucidate the complexity of Knops group system, the frequency of six single-nucleotide polymorphisms (SNPs) in three Brazilian populations is determined.

STUDY DESIGN AND METHODS: A total of 118 individuals descendant from Europe, Asia, and Africa were studied. The genomic fragment of CR1 was amplified by polymerase chain reaction, and the SNPs and haplotypes were determined after DNA sequence analysis.

RESULTS: Among the six polymorphisms characterized, one of them was described for the first time. The analysis of allele frequency showed that these six SNPs did not differ between the European and Asian groups. The African group presented a higher frequency of alleles McC^b, Sl2, and KAM+. The six polymorphisms gave origin to 12 haplotypes that were defined for the first time. The haplotypes 1 (4646A, Kn^a, McC^a, Sl1, Sl4, KAM+), 2 (4646A, Kn^a, McC^a, Sl1, KAM–), and 3 (4646A, Kn^a, McC^a, Sl2, Sl4, KAM–) are the most frequent in all populations. The H2 presents similar frequency in all populations; however, whereas the H1 presented a higher prevalence in the European and Asian groups, in the African group H3 was present in a higher prevalence.

CONCLUSIONS: In this study, a new SNP substituting serine for asparagine at amino acid 1540 was identified. Moreover 12 haplotypes were identified. The differences in haplotype frequencies strongly suggest that the H1 and H2 might be the ancestral one while the H3 may have originated in Africa and may have fixed there by positive selection.

The Knops blood group system is 22nd in the classification of the International Society of Blood Transfusion.[1] It consists of the isolated York antigen (Yk^a) and of the antithetic Knops antigens a and b (Kn^a, Kn^b), McCoy a and b (McC^a, McC^b), Swain-Langley/Vil (Sl1 and Sl2), and KAM– and KAM+, which was recently described.[2,3] A 10th antigen specificity denoted Sl3 and two new gene polymorphisms (Sl4/Sl5) have also been described.[2]

The antigens of the Knops system are located in the complement receptor type 1 protein (CR1 or CD35). CR1 is a constituent protein of red blood cell (RBC) membranes, macrophages, lymphocytes, dendritic cells, and renal podocytes. Its main function is the removal of immune complexes that present C4b and C3b. The extramembrane portion of CR1 is composed of 30 short consensus repeats (SCRs). Each SCR is composed of approximately 60 amino acids. These SCRs are further arranged into four long homologous regions having arisen by duplications of 7 SCRs.[4] The CR1 gene is located on the long arm of chromosome 1 (1q32). In addition to polymorphism of the Knops system, the CR1 gene presents two other polymorphisms. One is a structural polymorphism resulting from four different alleles that code for proteins with different molecular weights: 190 kDa (CR1*3), 220 kDa (CR1*1), 250 kDa (CR1*2), and 280 kDa (CR1*4). The

ABBREVIATIONS: CR1 = complement receptor 1; SCR(s) = short consensus repeat(s); SNP(s) = single-nucleotide polymorphism(s).

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CR1*1 allele is the most frequent and presents 38 exons. The CR1*2 allele presents 47 exons.[5] The third polymorphism correlates with quantitative expression of CR1 on RBCs, which is regulated by a genetic element that is linked to the site of a *Hin*dIII restriction fragment length polymorphism of the CR1 gene.[6] The alleles differ in having genomic *Hin*dIII fragments of 7.4 and 6.9 kb and are associated with either high (H allele) or low (L allele) expression of CR1, respectively.[6] Among normal individuals, the amount of CR1 on RBCs may differ up to 10-fold. RBCs with low expression levels (less than 10% of the normal value) have the "null" serologic phenotype known as Helgeson phenotype.[7]

The a and b Knops specificity is due to a G-A base change at position 4708 of exon 29 of the CR1 gene. This change determines the presence of valine at position 1561 of the Kn^a antigen and methionine in the Kn^b antigen.[8]

The McCoy specificity is also due to a single A-G base change at nucleotide 4795. This change determines the presence of valine at position 1590 of the McC^a antigen and glutamic acid in the McC^b antigen.[4]

The Swan-Langley/Vil antigens (Sl1/Sl2) are due to the A4828G nucleotide polymorphism, which determines the presence of arginine at position 1601 of the Sl1 antigen and glycine in the Sl2 antigen.[4]

A new A4855G gene polymorphism determines the presence of serine at position 1610 of the Sl4 antigen and threonine in the Sl5 antigen has been recently described.[2] So far, these two possible specificities have not been demonstrated serologically. The Sl3 specificity is due to the concomitant presence of Arg1601 (Sl1) and Ser 1610 (Sl4).[9]

The KAM–/KAM+ specificity is also due to a single A-G base change at nucleotide 4870. This change determines the presence of valine at position 1615 of the KAM+ antigen and isoleucine in the KAM– antigen. This KAM antigen has been recently described.[3]

CR1 is directly involved in the phenomenon of rosette formation between RBCs infected and noninfected with *Plasmodium falciparum.* Rosette formation is mediated by the PfEMP-1 receptor of the parasite (*P. falciparum* RBC membrane protein), which is present on the surface of infected RBCs and binds to CR1, among others, on the surface of noninfected RBCs.[10,11] RBCs with the Sl:1,2 phenotype form fewer rosettes than Sl:1,-2 RBCs. The Sl:1,2 phenotype is present at less than 1 percent frequency in Caucasian persons and at about 65 percent frequency in West African persons.[12] This polymorphism has been suggested to be selected in malarigenic areas because it confers protection against severe malaria.[12-14]

Brazil is a country of continental dimensions whose population has a high level of miscegenation between European, African, and native (Amerindian) populations. The objective of this study was to systematically sequence the region of exon 29 of the CR1 gene to determine the frequency of the polymorphisms of this exon in three different Brazilian populations and eventually identify new polymorphisms.

MATERIALS AND METHODS

Blood samples

The study was conducted on 118 persons of Brazilian descent, 33 persons of European descent, 43 persons of African descent, and 42 persons of Japanese descent. All participants reported the absence of racial-ethnic admixture in their grandparents and all resided in Ribeirão Preto and region (State of São Paulo). The study was approved by the Ethics Committee of FMRP-HCRP/USP, and all subjects gave written informed consent to participate.

Genomic DNA was isolated from 10-mL peripheral blood samples by the salting out technique with a DNA isolation kit (Super Quick-Gene, Analytical Genetic Testing Center, Denver, CO). After extraction and solubilization in water, the DNA was adjusted photometrically to the concentration of 100 ng per µL.

Polymerase chain reaction and DNA sequencing

Polymerase chain reaction (PCR) was applied for the molecular characterization of the polymorphisms of exon 29 of the CR1 gene with forward and reverse primers and a previously described protocol[4] with some modifications.

Briefly, primers 5′-TAAAAAATAAGCTGTTTTACCATA CTC-3′ (P5CR1) and 5′-CCCTCACACCCAGCAAAGTC-3′ (P3CR1) were used to amplify a 476-bp DNA fragment in a PCR containing 10 mmol per L Tris-HCl, 50 mmol per L KCL, 1.5 mmol per L $MgCl_2$, 0.2 mmol per L dNTP, 5 pmol of each oligonucleotide, and 0.5 U *Taq* DNA polymerase (Amersham-Pharmacia Biotech, Piscataway, NJ). The amplification reactions were carried out in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Foster City, CA) under the following conditions: 35 cycles of denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds and an extension at 72°C for 40 seconds, followed by a final extension cycle for 10 minutes at 72°C.

The amplification product was resolved on 1 percent agarose gel with 1× TAE buffer. The run was carried out at 8 V per cm at room temperature. The gel was then stained with 0.5 µg per mL ethidium bromide, visualized, and documented with an imaging instrument (Master VDS, Pharmacia Biotech, San Francisco, CA). The θX-174/*Hae*III marker (Promega, Madison, WI) was used to determine the size of the DNA fragment.

After amplification, the DNA fragments were purified with a PCR purification kit (QIAquick, Qiagen, Hilden, Germany) according to manufacturer instructions.

The DNA sequencing of the purified PCR products was carried out by the method of Sanger according to the protocol of a cycle sequencing ready reaction kit (Big Dye Terminator, Applied Biosystems), with some

modifications.[15] Briefly, approximately 90 ng of purified DNA was hybridized with 3.2 to 6.4 pmol of the oligonucleotide primer (P5CR1 or P3CR1) in a final volume of 10 µL to which 2 µL of the Big Dye kit buffer containing the AmpliTaq DNA polymerase enzyme and Big Dye terminators were added. The extension reactions were carried out in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems) under the following conditions: 35 denaturation cycles at 96°C for 30 seconds, annealing at 55°C for 20 seconds, and an extension at 60°C for 4 minutes. At the end of the reaction the extension products were precipitated with isopropanol, washed in the presence of 70 percent ethanol, and resuspended in 3 µL of running buffer (5 formamide:1 application buffer). The samples were denatured at 98°C for 2 minutes, and 1.2 µL was loaded onto 4 percent polyacrylamide gel and 6 mol per L urea under the following conditions: 1.0 kV, 35 mA, and 50 W, at 51°C for 8 hours with a DNA sequencer system (ABI Prism 377, Applied Biosystems). The sequences obtained were aligned with the accompanying computer program (ABI sequence program, version 3.1, Applied Biosystems). The sequences were then transferred to a Unix platform containing the Phred-Phrad-Consed package, which permitted the identification of single-nucleotide polymorphisms (SNPs) that were compared to the sequences deposited in GenBank (accession number Y00816).[16,17]

Data analysis

In the sequence analysis of the amplified genomic fragments of exon 29 of CR1N with the P5CR1 and P3CR1 oligonucleotides, only the 380 central bases of the fragment were considered. This portion was chosen because it presented satisfactory base resolution in all readings.

Haplotype definition

Haplotypes were defined manually, initially by analysis of the homozygous samples and next analyzing the heterozygous at single SNP. The 12 haplotypes were verified in the sequences and haplotypes named 1 through 12 in this study were deposited in GenBank under Accession Numbers AY701493 to AY701504.

Statistical analysis

The Turkey-Kramer test was applied for multiple comparisons of the populations according to the relative allele frequency or haplotype frequency, with computer software (GraphPad Instat, Version 2.05, GraphPad, San Diego, CA). The chi-square test was applied for the comparison of two populations with the same software. The level of significance was set at 0.5 percent for all tests.

TABLE 1. Genotypic frequency of six SNPs of exon 29 of the CR1 gene in three Brazilian populations

		Genotype (%)		
Polymorphism		European Brazilian (n = 33)	Asian Brazilian (n = 42)	African Brazilian n = 43
4646A>G	A/A	30 (91)	42 (100)	43 (100)
N1540S	A/G	3 (9)		
	G/G			
4708G>A	G/G	31 (94)	42 (100)	42 (98)
V1561M	G/A	2 (6)		1 (2)
Kn a/b	A/A			
4795A>G	A/A	32 (97)	42 (100)	25 (58)
K1590E	A/G	1 (3)		15 (35)
McC a/b	G/G			3 (7)
4828A>G	A/A	31 (94)	42 (100)	6 (14)
R1601G	A/G	2 (6)		24 (56)
Sl1/Sl2	G/G			13 (30)
4855T>A	T/T	32 (97)	42/100	43 (100)
S1610T	T/A	1 (3)		
Sl4/Sl5	A/A			
4870A>G	A/A	19 (57)	26 (62)	4 (9)
I1615V	A/G	12 (36)	14 (33)	19 (44)
KAM–/KAM+	G/G	2 (6)	2 (5)	20 (47)

RESULTS

Polymorphisms

Sequencing of the 476-bp fragment that codes for the SCR25 region of CR1 protein permitted the identification of six SNPs (Table 1). Five of these polymorphisms are associated with the Knops blood system (G4708A, A4795G, A4828G, T4855A, and A4870G) and have been previously described. The A4646G polymorphism is being described here for the first time.

Genotype frequency

The genotype frequency for the six SNPs studied in the three Brazilian populations are listed in Table 1. The Brazilian populations of European and Asian descent did not differ regarding the distribution of the genotypes analyzed. In these populations there was a predominance of the homozygote genotypes *4646A, Kn^a, McC^a, Sl1, Sl4,* and *KAM–*. The only polymorphism observed in the Asian-Brazilian population was at *KAM* site. In the Afro-Brazilian population, the 4646A and Sl4 sites did not show variation. The genotype frequencies for the *McC, Sl1,* and *KAM* polymorphisms differed between the Afro-Brazilian persons and the other two populations. We emphasize the prevalence of the *Sl2/Sl2* (30%) and *4870G/G* (47%) homozygote genotypes among Afro-Brazilian persons compared to the *Sl1/Sl1* (>97%) and *4870A/A* (>57%) homozygote genotypes in the Brazilian populations of European and Asian descent, respectively (Table 1). The *A4646G* polymorphism, described here for the first time, occurred only in

TABLE 2. Allele frequency of 6 SNPs of exon 29 of the CR1 gene in three Brazilian populations

Descent	4646 A>G N1540S	4708 G>A V1561M Kn a/b	4795 A>G V1590E McC a/b	4828 A>G R1601G Sl1/Sl2	4855 T>A S1610T Sl4/Sl5	4870 A>G I1615V KAM–/KAM+
European Brazilian	A 0.95	Kn^a 0.97	McC^a 0.98	Sl1 0.97	Sl4 0.985	A 0.76
Asian Brazilian	A 1.00	Kn^a 1.00	McC^a 1.00	Sl1 1.00	Sl4 1.00	A 0.79
African Brazilian	A 1.00	Kn^a 0.99	McC^a 0.76	Sl1 0.42	Sl4 1.00	A 0.32

TABLE 3. Haplotypes defined by six SNPs of exon 29 of CR1

			Haplotype					
Identifier	Number	Frequencies	N1540S 4646A>G	Kn^a/Kn^b V1561M 4708G>A	McC^a/McC^b V1590E 4795A>G	Sl1/Sl2 R1601G 4828A>G	Sl3/Sl4 S1610T 4855T>A	KAM–/KAM+ I1615V 4870A>G
H1	126	0.53	A	G	A	A	T	A
H2	41	0.17	A	G	A	A	T	G
H3	37	0.16	A	G	A	G	T	G
H4	10	0.04	A	G	G	A	T	G
H5	7	0.03	A	G	G	G	T	G
H6	6	0.025	A	G	G	G	T	A
H7	2	0.008	A	G	A	G	T	A
H8	2	0.008	A	A	A	A	T	G
H9	2	0.008	G	G	A	A	T	G
H10	1	0.004	G	G	A	A	T	A
H11	1	0.004	A	A	A	A	T	A
H12	1	0.004	A	G	A	A	A	G

TABLE 4. Haplotype frequency of six SNPs at exon 29 of CR1 in three Brazilian populations

	Haplotype*											
Group	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
European Brazilian	0.73	0.15	0.015		0.015			0.015	0.03	0.015	0.015	0.015
Asian Brazilian	0.79	0.21										
African Brazilian	0.14	0.15	0.42	0.12	0.07	0.07	0.023	0.011				

* Haplotypes 1 through 12 are deposited in Gene Bank under the following accession numbers: (1) AY701493; (2) AY701494; (2) AY701494; (2) AY701494; (3) AY701495; (4) AY701496; (5) AY701497; (6) AY701498; (7) AY701499; (8) AY701500; (9) AY701501; (10) AY701502; (11) AY701503; (12) AY701504.

the Euro-Brazilian population, with a predominance of the *4646 A/A* (91%) homozygote genotype.

Allele frequency

The allele frequencies for the six polymorphisms analyzed in the three Brazilian populations studied are listed in Table 2. Among Brazilian persons of European descent, the frequencies of *Kn^a, McC^a, Sl:1,-2, Sl4,* and *4646A* alleles were higher than 0.95 and did not differ from the frequencies observed in the Brazilian population of Asian descent ($p < 0.05$). The frequency of the *4870A* allele also did not differ between these two populations (0.76 vs. 0.79; $p > 0.05$).

In the Afro-Brazilian population, the frequencies of *4646A, Kn^a,* and *Sl4* alleles were higher than 0.99 and did not differ from those observed in the other two populations. The frequencies of *McC^a, Sl1,* and *4870A* alleles in this population, however, were significantly lower than those observed in the populations of European or Asian descent ($p < 0.001$).

Haplotypes

In the three populations studied, the six SNPs gave origin to 12 haplotypes (Table 3). Only the H1 and H2 haplotypes were observed in the Asian-Brazilian population (Table 4).

Nine of the 12 haplotypes described were detected among the Euro-Brazilian population. The H1 and H2 haplotypes, however, represent 88 percent of all haplotypes detected in this population. The H9, H10, H11, and H12 haplotypes were detected only in this population, at low frequency.

Eight haplotypes were observed in the Afro-Brazilian population, with the H1 to H6 haplotypes representing 97 percent of all haplotypes of this population. The H3, H4, H5, H6, and H7 haplotypes were more frequent in this population, with the H4, H6, and H7 haplotypes being exclusively detected in it.

The HI haplotype was more frequent in the Euro-Brazilian (0.73) and Asian-Brazilian (0.79) populations than in the Afro-Brazilian one (0.14; $p < 0.0001$; Table 4). The H2 haplotype occurred at similar frequencies in the three populations ($p = 0.3$) and the H3 haplotype was the most frequent in the Afro-Brazilian population ($p < 0.001$).

DISCUSSION

In this study we determined the nucleotide base sequences of exon 29 of the CR1 gene in 118 Brazilian individuals of European (n = 33), Japanese (n = 42), and African (n = 43) descent. In addition to the detection of five polymorphisms associated with the Knops blood system, we detected the occurrence of a new A4646G polymorphism. These six SNPs were organized into 12 haplotypes that are described here for the first time (Tables 3 and 4).

The *G4708A* polymorphism is responsible for the determination of the alleles that code for the Kn^a/Kn^b antigens. In the three populations studied, the frequency of the Kn^a allele was higher than 0.97. Heterozygous Kn^a/Kn^b individuals were identified only in populations of European (2/33) and African (1/43) descent. The frequencies of this allele in the Brazilian populations did not differ from those detected in Caucasian American persons (0.99), African American persons (0.98), and African persons from Mali (0.90).[8]

The McC^a antigen was first described in association with the Kn^a antigen.[18] This association occurred in 95 percent of Euro-Brazilian individuals, in 100 percent of Asian-Brazilian individuals, and in 70 percent of Afro-Brazilian individuals. The McC^a allele was observed at lower frequency (0.76) among Afro-Brazilian persons than among Euro-Brazilian (0.98) and Asian-Brazilian persons (1.00). These allele frequencies did not differ from those observed in Caucasian American (1.00), Asian-American (0.96), African American (0.78), West African (0.69), and Mali African persons (0.70).[4] The frequency of the McC^a allele observed among Afro-Brazilian persons, however, was higher than that observed among Black persons from Gambia (0.39; $p = 0.008$).[13] These findings can be explained from our African-Brazilian history during the Atlantic slave trade. According to these studies between 1701 and 1810 Brazil received approximately 2 million slaves of which 70 percent originated from Bantu-speaking Africa (Angola, Congo, and Mozambique), 25 percent from Central West Africa (Bight of Benin and Bight of Biafra), and a small group (5%) from Atlantic West Africa (Senegambia, Guinea-Bissau, and Cape Verde).[19] In fact, previous populational studies conducted first by our group[20,21] and confirmed recently by another research group[22] about the origin of Afro-derived populations on the basis of βS haplotypes have demonstrated the predominance of the Bantu (Angola, Congo, and Mozambique) haplotype, followed by Benin and Senegal (Senegambia, Guinea-Bissau, and Cape Verde) haplotype. All together, these data corroborate with the formation of our African descendent population which has been suggested that groups from Angola, Congo, and Mozambique brought a major contribution in comparison with people from Gambia.

Serologic studies have shown that the Sl1 antigen appears at high frequency among white persons and at a characteristically low frequency among blacks.[23] In agreement with this initial observation, the frequency of the *Sl:1,-2* allele was higher among Brazilian persons of European (0.97) and Asian (1.00) origin than among Afro-Brazilian persons (0.42). The frequency of the *Sl:1*,-2 allele observed among Afro-Brazilian persons did not differ from the frequency observed among African American persons (0.47; $p = 0.47$),[1] but was higher than that observed among West African (0.21; $p < 0.002$) and Mali African persons (0.24; $p < 0.01$).[4]

The T4855A polymorphism is responsible for the determination of the *Sl4/Sl5* alleles. The frequency of the SI4 allele was higher than 0.97 in all three populations studied and did not differ from the frequency observed in Caucasian (0.96), African black (1.00), and Asian-American persons (0.99).[2]

The occurrence of the SI3 serologic specificity as due to the concomitant presence of the Sl1 and Sl4 antigens has been recently reported.[1,4] Among white individuals, the most common phenotype may be Sl:1,-2,3, as opposed to Sl:=1,Sl2,Sl=3 among blacks. In this study, all Asian-Brazilian individuals and 94 percent of Euro-Brazilian individuals showed the concomitant presence of Sl1 and Sl4, thus presenting the Sl3 (Sl:1,-2,3) specificity. In contrast, only 28 percent of the Afro-Brazilian persons presented this association, implying that 72 percent of these individuals may be Sl-3 (Sl:=1,Sl2,Sl=3). Among the 12 haplotypes described in Table 3, the H1, H2, H4, H8, H9, H10, and H11 haplotypes could determine the SI3 specificity. Except for H4 and H8, the remaining haplotypes predominate among Euro-Brazilian and Asian-Brazilian persons.

At position 4870 of exon 29 of the CR1 gene, the A/G polymorphism determines a change of Ile at position 1615 to Val in the protein.[5,6] Recent studies conducted with this polymorphism showed that it is related with KAM+/KAM– antigens.[3] The three Brazilian populations studied here showed high frequencies of this polymorphism, a fact that may be of evolutionary significance. The *A4870* allele (KAM+) was more frequent in the populations of European (0.76) and Japanese (0.79) descendants than in the Afro-Brazilian population (0.32). The high frequency of the *G4870* allele (KAM–) in the Afro-Brazilian population may indicate that this allele, in the same way as the *Sl:=1,2* allele, may have been selected by evolutionary pressure.[13] It has been suggested that the pattern of world distribu-

tion of *McC^a/Sl1* alleles and the presence of the *McC^b/Sl2* alleles limited to African populations and their descendants may indicate that the *McC^a/Sl:=1,2* alleles may be the ancestral ones and that the *McC^b/Sl:1,2* alleles may have specifically arisen in African populations, becoming fixed there by selective pressure.[13]

The *McC^b* allele is part of the composition of the H4, H5, and H6 haplotypes. The H4 haplotype is associated with the *Sl:1,-2* and 4870G alleles and the H5 and H6 haplotypes are associated with *Sl:=1,2* and with *4870G* and *4870A*, respectively. We detected four Afro-Brazilian individuals with the *McC^b* allele in homozygosis, two of them with the H5 haplotype and two with the H6 haplotype, indicating lack of dependence of A4870G polymorphism regarding the *McC^b* and *Sl:=1,2* alleles.

In contrast, the *Sl:=1,2* allele was found in homozygosis in 13 Afro-Brazilian individuals, but not in any individual of European or Japanese descent. The Sl:=1,2 allele is part of the composition of the H3, H5, H6, and H7 haplotypes. Of these *Sl:=1,2/Sl:=1,2* individuals, 4 were homozygotes for H3 haplotype, 2 were homozygotes for H5 haplotype, 2 were homozygotes for H6 haplotype, and the remaining ones were H3/H5 (2), H3/H7 (2), and H3/H6 (1) heterozygotes. The H3 haplotype was the most frequent in the Afro-Brazilian population (0.42) and occurred only in heterozygosis in 2 Euro-Brazilian individuals. It is interesting to note that haplotype 3 is also composed of *McC^a* and *4870G* alleles. Therefore, this result clearly shows that the *McC^a/Sl:=1,2* (haplotypes H3 and H7) and *Sl:=1,2/4870G* (haplotypes H3 and H5) associations were the most frequent among Afro-Brazilian persons. The H4, H6, and H7 haplotypes occurred exclusively in the Afro-Brazilian population and can be considered to be markers of this population. The exclusivity of African occurrence and high frequency of H4 haplotype (0.12) indicates that this haplotype may have originated in Africa after the dissemination of ancestral haplotypes and may have fixed there by positive pressure. A similar hypothesis was raised regarding the *McC^b/Sl:=1,2* haplotype (haplotypes H5 and H6) whose frequency is high in the population of Gambia living in a malarigenic zone.[13] Similarly, it is interesting to note the high frequency of the 4870G allele in the Afro-Brazilian population, which may also have been selected by positive pressure. Studies involving the sequencing of exon 29 of the CR1 gene and case-control studies conducted on other populations are needed to clarify these questions.

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