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ß^S-Haplotypes in sickle cell anemia patients from Salvador, Bahia, Northeastern Brazil

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Abstract

ß^S-Globin haplotypes were studied in 80 (160 ß^S chromosomes) sickle cell disease patients from Salvador, Brazil, a city with a large population of African origin resulting from the slave trade from Western Africa, mainly from the Bay of Benin. Hematological and hemoglobin analyses were carried out by standard methods. The ß^S-haplotypes were determined by PCR and dot-blot techniques. A total of 77 (48.1%) chromosomes were characterized as Central African Republic (CAR) haplotype, 73 (45.6%) as Benin (BEN), 1 (0.63%) as Senegal (SEN), and 9 (5.63%) as atypical (Atp). Genotype was CAR/CAR in 17 (21.3%) patients, BEN/BEN in 17 (21.3%), CAR/BEN in 37 (46.3%), BEN/SEN in 1 (1.25%), BEN/Atp in 1 (1.25%), CAR/Atp in 6 (7.5%), and Atp/Atp in 1 (1.25%). Hemoglobin concentrations and hematocrit values did not differ among genotype groups but were significantly higher in 25 patients presenting percent fetal hemoglobin (%HbF) ≥10% (P = 0.002 and 0.003, respectively). The median HbF concentration was 7.54 ± 4.342% for the CAR/CAR genotype, 9.88 ± 3.558% for the BEN/BEN genotype, 8.146 ± 4.631% for the CAR/BEN genotype, and 4.180 ± 2.250% for the CAR/Atp genotype (P = 0.02), although 1 CAR/CAR individual presented an HbF concentration as high as 15%. In view of the ethnic and geographical origin of this population, we did not expect a Hardy-Weinberg equilibrium for CAR/CAR and BEN/BEN homozygous haplotypes and a high proportion of heterozygous CAR/BEN haplotypes since the State of Bahia historically received more slaves from Western Africa than from Central Africa.

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- Fetal hemoglobin
- Sickle cell anemia
- S hemoglobin
- Brazilian population

Introduction

Sickle cell hemoglobin (HbS) is the result of a single nucleotide change (GAG→GTG) in the ß-globin gene, where valine replaces glutamic acid (ß$^{6\ Glu\rightarrow val}$) at the sixth amino acid position of the ß-globin chain (1). Sickle cell anemia is caused by homozygosity of the ß^S-gene and has a worldwide distribution. The disease progresses as severe chronic hemolytic anemia, presenting a heterogenous clinical course according to patient back-

ground and geographic region of origin (2). Milder clinical symptoms have been described in patients presenting α-2 thalassemia and high levels of fetal hemoglobin (HbF), related to the presence of specific haplotypes (3,4). $ß^S$-Haplotypes are of different ethnic and geographic origins: the Benin type (BEN) originated in Midwestern Africa, the Bantu (CAR) type in South-Central and Eastern Africa, the Senegal (SEN) type in Atlantic West Africa, the Saudi Arabia-India type on the Indian subcontinent and the eastern Arabian peninsula, and the Cameroon type along the west coast of Africa (5).

Sickle cell disease affects millions worldwide, and occurs in one of every 500 African-American births, and in one of every 1000 to 4000 Hispanic-American births. In Brazil, the largest country in South America, the sickle cell trait is found at frequencies ranging from 6.9 to 15.4% of individuals of African descent (6). High immigration influxes have produced a population of significant cultural, social, and ethnic heterogeneity. Salvador is the capital of Bahia, a state in the Northeast region of Brazil, with 2.7 million people (7). The population has a high racial admixture with 85% of the African component (8). Historical data describing the slave trade in Bahia indicate the presence of slaves from central Africa (predominantly CAR haplotype) and from Western Africa (BEN haplotype), with a predominance of the latter. However, haplotype characterization has reported conflicting frequencies of CAR (9) and BEN (10) haplotypes.

The historian Verger (11) described the Nagô-Iorubá influence in Bahia State brought by Africans from the Benin Gulf region. In contrast, the rest of Brazil received large influxes of slaves from Congo and Angola (primarily CAR haplotype). In addition to a period of illicit slave trafficking, Bahia had four official periods of slave trading: a Guinea cycle during the XVI century, an Angola and Congo cycle during the XVII century, a Coast of Mine cycle during the XVIII century, and a Bay of Benin cycle between 1770 and 1850. Florentino (12) emphasizes that Bahia, beginning in 1815, was the only Brazilian state that restricted slave traffic through Ecuador, a fact that explains the correlation between genotype frequencies found in Bahia and Western Africa, principally the Bay of Benin region.

The unusual ethnic composition of Salvador, which was a transfer point during the African slave trade, represents an excellent opportunity to study the $ß^S$-haplotypes and to investigate the clinical picture of sickle cell anemia patients and the anthropological origins of the $ß^S$-gene in this Brazilian population.

Material and Methods

A total of 80 sickle cell disease patients (40 males and 40 females) were studied. Informed consent was obtained from all individuals or responsible person prior to enrollment and the study protocol was submitted to and approved by the FIOCRUZ Ethics Committee. Patients were recruited from both the Center for Hematological Studies (Fundação Hemocentro da Bahia, HEMOBA) and the University Hospital, Federal University of Bahia (Hospital Universitário Professor Edgar Santos, Universidade Federal da Bahia). Mean patient age was 13.17 ± 9.71 years (range: 1.6-51.5 years).

Hematological analyses were carried out using an electronic cell counter (Coulter Count T890). Hemoglobin type was determined by electrophoresis on cellulose acetate strips at pH 8.4, and the presence of HbS was confirmed by sickling and solubility tests, and by electrophoresis on agar-citrate at pH 5.3 (13). HbF was measured by alkali denaturation (13). DNA was isolated from peripheral blood leukocytes (14). $ß^S$-Haplotypes were established by PCR and by dot-blot methods that characterize DNA polymorphisms of the 5' flanking region and the

second intervening sequence (IVS-II) of the γ-globin genes (15,16) (Figure 1).

The EPI Info (version 6.04) and Statistical Package for the Social Sciences (SPSS, version 6.1) programs were used for statistical analyses. The effects of age category, gender, and HbF concentration ≥10% on the hematological parameters were evaluated. The level of significance was set at $P < 0.05$ in all analyses.

Results

The patients had a median (± SD) hemoglobin concentration of 8.369 ± 1.632 g/dl, median hematocrit of 25.044 ± 5.03%, median cell volume of 88.488 ± 10.033 fl, median cell hemoglobin of 30.095 ± 4.195 pg, and median cell hemoglobin concentration of 33.905 ± 1.679 g/dl. These parameters did not vary significantly between age and gender categories. However, patients with HbF ≥10% were found to have significantly higher Hb concentrations compared to patients of the group with Hb <10% (median: 7.8 *vs* 9.0; P = 0.002) and hematocrit values (median: 24.00 *vs* 28.00; P = 0.003).

The hematological data and the ßS-haplotypes/genotypes obtained for the 80 sickle cell disease patients analyzed are listed in Table 1.

The hematological data, including the different proportions of HbF found, are reported in Table 2. Median age was significantly higher among the CAR/CAR and CAR/BEN genotypes. The median HbF levels among the CAR/CAR, CAR/BEN, BEN/BEN and CAR/atypical (Atp) genotypes are shown in Figure 2. The patient group presenting HbF ≥10% consisted of 25 (31.2%) individuals; the genotype was CAR/BEN in 12, CAR/CAR in four, BEN/BEN in seven, SEN/Atp in one, and Atp/Atp in one. There was no CAR/Atp or BEN/SEN genotype in this group. In the group with HbF higher than 10%, eight subjects presented HbF ≥15%, with the genotype being CAR/CAR in one of

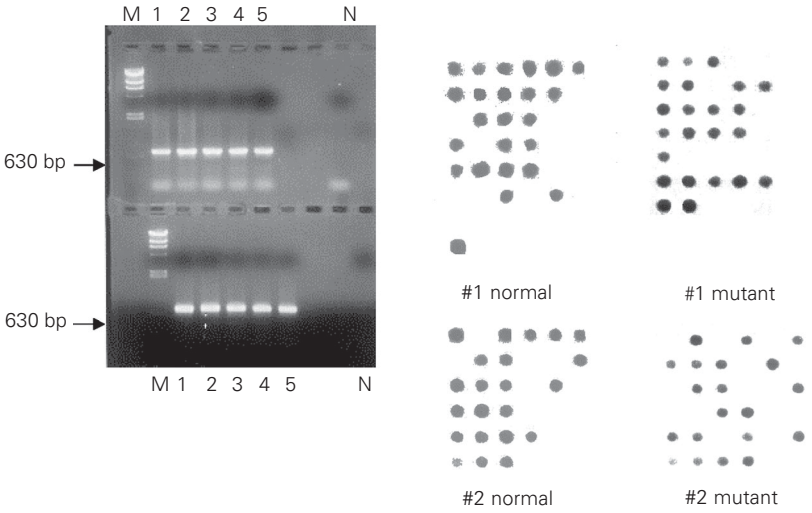

Figure 1. Gamma-globin gene amplification and dot-blot analyses of CAR ßS-haplotype. Sickle cell disease patients, heterozygous for the CAR haplotype, have a positive signal with normal and mutant probes. Homozygous patients have a positive signal only with a mutant probe and negative patients for this haplotype have a signal only with a normal probe. Lanes 1-5 show a 630-bp PCR fragment from the Gγ-globin. M = λ *Hin*dIII marker; N = negative control.

Table 1. Hematological data of the 80 sickle cell disease patients (40 males and 40 females) from Salvador, Bahia, Brazil, and ßS-haplotype frequencies and genotype frequencies found among the 80 sickle cell disease patients.

	Mean ± SD (median)
Age (years)	13.179 ± 9.715 (10.55)
Hematological data	
Ht (%)	25.044 ± 5.030 (24.00)
Hb (g/dl)	8.369 ± 1.632 (8.300)
MCV (fl)	88.488 ± 10.033 (91.00)
MCH (pg)	30.095 ± 4.195 (30.00)
MCHC (g/dl)	33.905 ± 1.679 (34.00)
HbF (%)	8.253 ± 4.636 (8.20)
HbS (%)	89.876 ± 4.476 (90.00)
Haplotypes/genotypes	
CAR/CAR	17 (21.25%)
BEN/BEN	17 (21.25%)
CAR/BEN	37 (46.25%)
CAR/Atp	6 (7.5%)
BEN/Atp	1 (1.25%)
BEN/SEN	1 (1.25%)
Atp/Atp	1 (1.25%)

Hb = hemoglobin; HbF = fetal hemoglobin; HbS = sickle cell hemoglobin; Ht = hematocrit; MCH = median cell hemoglobin; MCHC = median cell hemoglobin concentration; MCV = median cell volume; Atp = atypical; BEN = Benin; CAR = Central African Republic; SEN = Senegal.

Table 2. Characterization of patients with the CAR/CAR, BEN/BEN, CAR/BEN and CAR/Atp genotypes.

	CAR/CAR (N = 17)	BEN/BEN (N = 17)	CAR/BEN (N = 37)	CAR/Atp (N = 6)
Age	16.72 ± 12.89 (11.05)	8.213 ± 4.651 (6.70)	14.34 ± 9.904 (10.60)	7.267 ± 1.193** (7.80)
Gender (N, %)				
Male	10 (58.8%)	8 (47.0%)	17 (45.9%)	3 (50.0%)
Female	7 (41.2%)	9 (53.0%)	20 (54.1%)	3 (50.0%)
Hematological data				
Ht (%)	23.67 ± 2.934 (23.50)	24.44 ± 5.49 (24.50)	26.00 ± 5.82 (27.00)	23.667 ± 3.79 (22.00)
Hb (g/dl)	7.88 ± 0.824 (7.950)	8.15 ± 1.89 (8.350)	8.79 ± 1.79 (8.80)	7.867 ± 1.03 (7.600)
MCV (fl)	86.29 ± 8.18 (88.00)	90.54 ± 12.39 (91.00)	86.78 ± 9.76 (87.50)	90.33 ± 7.23 (94.00)
MCH (pg)	29.00 ± 3.21 (29.50)	30.91 ± 4.99 (32.00)	29.72 ± 4.43 (30.00)	30.33 ± 3.79 (32.00)
MCHC (g/dl)	33.25 ± 1.49 (33.50)	34.18 ± 1.08 (34.00)	34.11 ± 2.11 (34.00)	33.33 ± 1.53 (33.00)
HbF (%)	7.544 ± 4.342 (7.350)	9.882 ± 3.558 (9.600)	8.146 ± 4.631 (7.800)	4.180 ± 2.250* (4.000)
HbS (%)	90.434 ± 4.01 (90.530)	88.010 ± 3.66 (88.170)	90.005 ± 4.51 (90.44)	94.432 ± 2.10* (94.490)

Data are reported as means ± SD (median). Atp = atypical; BEN = Benin; CAR = Central African Republic. For other abbreviations, see legend to Table 1.
*P < 0.05 for HbF and HbS concentrations between the different genotypes (Kruskal Wallis test). **P < 0.05 for age between the different genotypes (ANOVA + chi-square test).

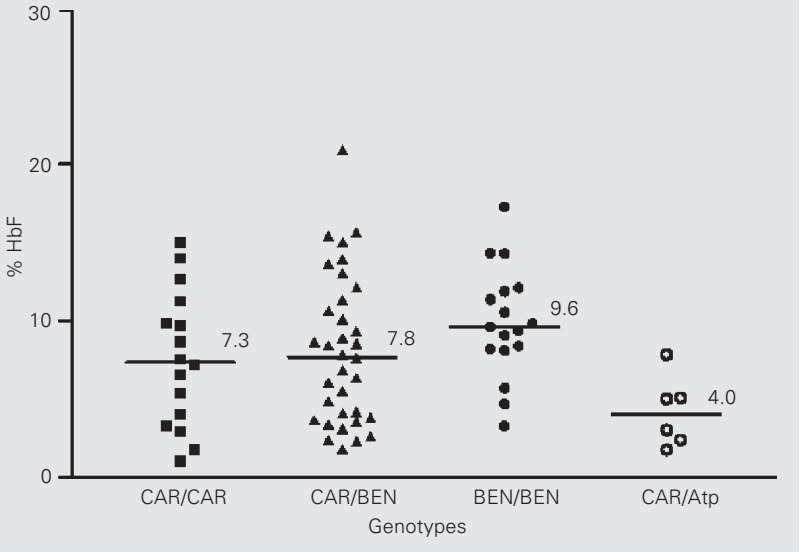

Figure 2. Distribution of median fetal hemoglobin (HbF) levels among the CAR/CAR (N = 17), CAR/BEN (N = 37), BEN/BEN (N = 17) and CAR/Atp (N = 6) genotypes in sickle cell disease patients from Salvador, Bahia, Brazil. For abbreviations, see legend to Table 2.

them, CAR/BEN in four, BEN/BEN in one, SEN/Atp in one, and Atp/Atp in one. Median HbS values were higher among the subjects with the CAR/Atp genotype and lowest among subjects with the BEN/BEN genotype.

Discussion

ß^S-Haplotypes were established in 80 sickle cell disease patients from Salvador, a city in Northeastern Brazil characterized by a population with a large African contribution (8). Azevedo et al. (6) found that the frequency of HbS ranged from 7.6 to 15.9% in different population groups of Salvador. In the present study, the CAR/BEN genotype was predominant. Unexpectedly, the BEN and CAR homozygous genotypes were found to occur at similar frequencies, mainly considering the high presence of the CAR haplotype. Verger (11) emphasized that from 1678 to 1814, only 39 of 1770 ships that exported tobacco from Bahia went to the Congo and Angola, where they captured slaves representing a possible contribution of Africans from Atlantic Central Africa. All the other ships went to Coast of Mine ports. The slave traffic from Atlantic Central Africa was supposedly intensified between 1815 and 1824 (11), a fact that can explain our results. No Saudi Arabian or Cameroon haplotypes were identified in the study sample, and only one SEN haplotype was encountered.

In the United States and Jamaica, the BEN haplotype is predominant, a result of the preference for the traffic of Midwestern Africans to these regions during the British Atlantic slave trade (4,17,18). In contrast, haplotype studies on the Cuban and Puerto Rican populations have found a predominance of genes from the Bantu haplotype, suggesting a different African origin of these populations (5,19-21).

The distribution of ß^S-haplotypes in the Brazilian State of São Paulo (Southeastern

Brazil) and Pará (Northern Brazil) showed high frequencies of the CAR haplotype, i.e., 62.2 and 65.9%, respectively (9,22-24). Analyses of ßS-haplotypes from the Amazon region have indicated a 60% frequency of the CAR haplotype, a 30% frequency of the SEN haplotype, and a 10% frequency of the BEN haplotype (24).

Populations with a high frequency of BEN/CAR heterozygotes, as reported for Bahia, provide an excellent cohort for the study of the effect of ßS-haplotypes on the clinical course of sickle cell anemia. An important finding of the present study was the high concentration of HbF among individuals with the CAR/CAR genotype, which normally present a median HbF value below 5.0% (4). It is well known that HbF levels in sickle cell anemia could be influenced by age, gender, α-globin gene number, ß-globin haplotype, and the X-linked F-cell production locus that regulates the production of HbF-containing erythrocytes (F cells) (25).

In a previous study, the F-cell production locus accounted for 40% of the overall variation of HbF levels and the ß-globin haplotype was associated with 14% of the remaining HbF variation; when the F-cell production influence was removed, approximately half of the variation in HbF levels still remained to be explained, showing the need for further studies (25). Unfortunately, we did not study α-thalassemia in theses patients, but a higher frequency of this type of thalassemia was previously demonstrated among Bahian sickle cell disease patients (10), probably representing an important prognostic factor for the clinical course of the disease.

The presence of high HbF levels in the CAR/CAR genotype could be explained by sequence variation in regulatory regions of the 5' HS2 and 5' flanking region of the γ-gene expression, as previously discussed by Lanclos et al. (26).

In addition, we also identified an individual with the SEN haplotype, a fact that may suggest that Bahia State also had a gene flow from Atlantic West Africa, as was the case for other Brazilian states (24). Internal migration is unlikely since the patient's ancestors were from Salvador. The low frequency of the SEN haplotype could be explained by the absence of SEN carriers looking for medical care or a recent origin of the ßS SEN mutation in this population (27). The atypical haplotypes showed different distributions and could be found associated with the CAR and BEN genotypes, indicating the occurrence of diverse genetic mechanisms that could be responsible for the variation of HbF concentrations among the atypical haplotype carriers (28-30).

The present results are relevant to the study of slave traffic routes in Brazil and of the African origins of the Bahian population. Taken together, the data indicate that studies examining the impact of the ßS-haplotypes and of the presence of α-thalassemia on clinical outcome and HbF expression may contribute to clarifying a possible relationship between clinical picture, ßS-haplotypes, α-thalassemia and HbF production in sickle cell disease patients from Salvador, Bahia, identifying prognostic factors for the clinical course of the disease in this population.

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