

## Beta-Globin Gene Cluster Haplotypes as Evidence of African Gene Flow to the Northeastern Coast of Venezuela

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**ABSTRACT** In order to study the origin of mutation HBB\*S in Sucre and Anzoátegui states and the genetic affinities of these Venezuelan populations with other human groups, the  $\beta$ -globin gene cluster haplotypes were determined for 28 sickle cell and/or S-beta thalassemia patients and for 37 individuals with normal hematological parameters. Bantu, Benin, Senegal, and atypical haplotypes were identified in 50%, 36%, 2%, and 12% of the HBB\*S chromosomes, respectively. Similar results have been published for Venezuelan patients from the central states, but a different trend is shown in a publication based on a group of patients from different regions of the country. For HBB\*A, haplotype 2 (+ - - -), characteristic of non-African groups, was the most common (39%), followed by haplotype 3 (- - - +) of African origin, and haplotype 6 (- + + -), also typical of non-Africans. The results reveal a high level of admixture of the Sucre–Anzoátegui population. The importance of specific conditions which have acted differently in the Venezuelan populations, such as founder effect, genetic drift, isolation, and endogamy are discussed. Genetic distances between the Sucre–Anzoátegui sample and several other human populations calculated on the basis of the HBB\*S and HBB\*A haplotypes revealed similar results, the closest genetic relationships being observed in relation to Bantu-speaking groups. These results confirm the utility of the  $\beta$ -globin haplotypes for population studies and contribute to knowledge of the Venezuelan gene pool. *Am. J. Hum. Biol.* 15:29–37, 2003. © 2002 Wiley-Liss, Inc.

The HBS mutation ( $\beta 6$  Glu→Val) has been associated with five main haplotypes usually defined by six polymorphic restriction endonuclease sites in and around the  $\beta$ -like globin gene: Benin (BEN: - - - - + -), Central African Republic or Bantu (CAR or BAN: - + - - - -), Senegal (SEN: - + - + + +), Cameroon (CAM: - + + - + +) and Arab-Indian (ARB: + + - + + -), which are probably related to the clinical heterogeneity of sickle cell anemia (Nagel et al., 1987, 1991; Elion et al., 1992). The presence of these haplotypes can also suggest the place of origin of mutation HBB\*S in the population under study (Gonçalves et al., 1994; Steinberg et al., 1997; Pante de Sousa, 1999).

On the other hand, analysis of haplotypes associated with the normal allele, HBB\*A, are useful in studies of genetic diversity and interpopulation relationships. Wainscoat et al. (1986) were the first to carry on this type of investigation using RFLPs in the  $\beta$ -globin gene cluster. Subsequently, Long et al. (1990) and Chen et al. (1990) revealed that Africans can be distinguished from non-Africans through distributions of five haplotypes: 2 (+ - - -), 3 (- - - -),

4 (- + - - +), 5 (- + - + +), and 6 (- + + - +). Haplotypes 3 and 4 have been considered common genetic markers of African populations, while haplotype 2 is characteristic of non-Africans.

Allele HBB\*S was introduced into Venezuela by migratory waves from Africa during the slave trade. HBB\*S is more frequent among African descendants, who inhabit an extensive coastal region from the East to the West of the country (Acosta Saignes, 1969; Castro de Guerra, 1992). Molecular studies of the  $\beta$ -globin gene clusters in Venezuelans are scarce, but the predominance of the Bantu haplotype (47%) has been reported in patients with sickle cell anemia from Carabobo and Cojedes states and from the central-western region,

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while in patients from Venezuela as a whole the Benin haplotype is predominant, 51% (Olivero, 1966; Arends et al., 2000). Considering the HBB\**A* allele, high frequencies have been reported for haplotypes 2 (+ - - - -) and 3 (- - - - +) in Maracay (Aragua state) and Panaquire (Miranda state), suggesting an important admixture component in these populations (Olivero et al., 1996; Castro de Guerra et al., 1997).

This study presents the haplotype frequencies related to the HBB\**S* and HBB\**A* alleles in patients and normal individuals from the coastal region of Anzoátegui and Sucre states and from eastern Venezuela. The most probable origin of the HBB\**S* allele in these geographical areas, as well as the level of the intrapopulation haplotype diversity and interpopulation genetic relationships, are also considered.

## SUBJECTS AND METHODS

Forty-two chromosomes with mutation HBB\**S* from 28 sickle cell anemia or S/ $\beta$ -thalassemia patients were studied. All patients were attending clinics of the Hematology Department of the hospitals Antonio Patricio Alcalá and Luis Razetti in Cumaná, Sucre state (10° 0', 10° 45'N, and 61° 50', 64° 30'W), and Barcelona, Anzoátegui state (7° 45', 10° 15'N, and 62° 30', 65° 45'W), respectively (Fig. 1). There were 22 females (79%) and 6 males (21%) with ages between 11 months to 41 years. In addition, 37 individuals with normal hematological parameters (homozygous for HBB\**A*) were also studied. Patients or their parents and normal individuals, referred to as the Sucre-Anzoátegui sample, gave informed consent to participate in this study. None of

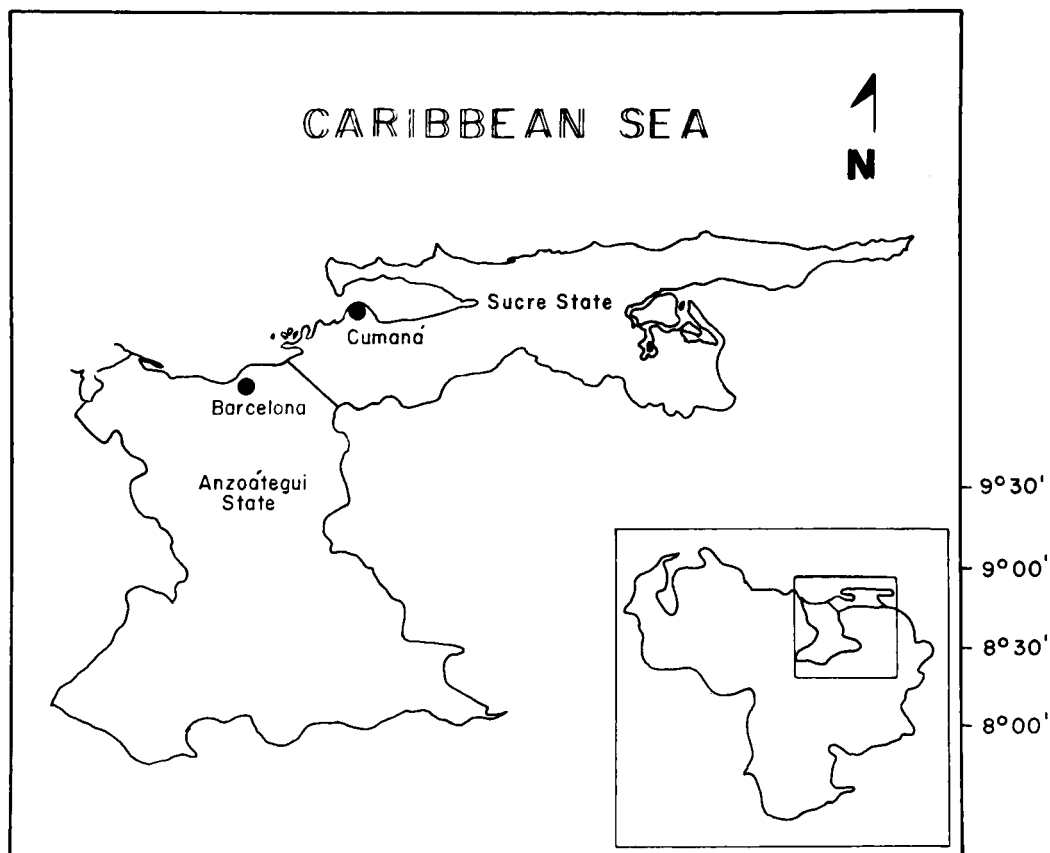


Fig. 1. Geographical location of Sucre and Anzoátegui states. The lower right map of Venezuela indicates the region considered.

subjects were known to be biologically related and their parents and grandparents were originally from the two states.

DNA was isolated from 3 ml of whole blood according to procedures described by Lahiri and Nurnberger (1991) and Rudbeck and Dissing (1998). Specific regions of  $\beta$ -globin gene cluster, located on the short arm of chromosome 11, were amplified by PCR using primers and conditions described previously (Guerreiro et al., 1992; Li et al., 1985; Saiki et al., 1988; Sutton et al., 1989). PCR modifications were done in order to have a mixture with a final volume of 15  $\mu$ l. The amplified segments were then digested with corresponding restriction enzymes as follows: 1) Hinc II, 5' of gene HBE; 2) Hind III, in IVS-2 of gene HBGG ( $\gamma^G$ ); 3) Hind III in IVS-2 of gene HBGA ( $\gamma^A$ ); 4) Hinc II in pseudo-gene HBBP1 ( $\psi\beta$ ); 5) Hinc II in the 3' region of HBBP1 (3' $\psi\beta$ ); and 6) Hinf I in the 5' region of gene HBB (5' $\beta$ ). The fragments were separated by PAGE 8% and stained with silver nitrate. DNA of plasmid pBR 322 digested with the restriction enzyme Msp I was used as molecular weight marker. Haplotypes associated with the mutation (HBB\*S) and those associated with the normal allele (HBB\*A) were built as reported by Pagnier et al. (1984) and Nagel et al. (1985). Family studies were not done for economic reasons. Briefly, it assumes as more probable the presence of two common haplotypes or the combination of a common and a rare one, instead of two rare haplotypes (Kulozik et al., 1986; Long et al., 1990; Rieder et al., 1991; Konstantopoulus et al., 1996; Olivero, 1996; Castro de Guerra et al., 1997).

Haplotype frequencies were estimated by the counting method, while intrapopulation haplotype variability and Hardy-Weinberg equilibrium were obtained with the SATFILES program (Dr. I. Barrai, University of Ferrara, Italy). The DISPAN computer

program (Ota, 1993) was used to calculate Nei's genetic distances (Nei et al., 1983; Nei and Roychoudury, 1993) between the present sample and other populations. The corresponding dendrogram was obtained using the neighbor joining method (Saitou and Nei, 1987).

## RESULTS

Haplotype frequencies associated with HBB\*S are given in Table 1. Among the patients, 51% (21 chromosomes), 36% (15), 2% (1), and 12% (5) present the Bantu, Benin, Senegal, and atypical haplotypes, respectively. Table 2 furnishes in increasing order the genetic distances based on haplotypes associated with HBB\*S between Sucre-Anzoátegui and 34 populations around the world, including two other Venezuelan samples. The Sucre-Anzoátegui shows the smallest genetic distance ( $\sim 0.009$ ) with Carabobo-Cojedes, another sample from Venezuela. Populations identified as Afro-American from Mexico, Surinam, Brazil, and Cuba also present an important level of identity with Sucre-Anzoátegui. Among Africans, the populations most related to Anzoátegui-Sucre are those from central and south (Mozambique, St. Tome, and Prince and Central African Republic) Africa, while those from the eastern central area (Benin, Guinea-Bissau, Senegal, and Guinea Republic) show the largest distances. Table 2 also shows the corresponding levels of intrapopulation haplotype diversity for these samples. Sucre-Anzoátegui shows one of the highest levels of intrapopulation haplotype diversity (62%).

The dendrogram based on genetic distances in Table 2 is given in Figure 2. The Sucre-Anzoátegui cluster with other American, Caribbean, and African populations.

TABLE 1. Frequencies of  $\beta$ -globin gene cluster haplotypes in sickle cell anemia patients from Sucre-Anzoátegui

Haplotypes	Restriction sites						N <sup>1</sup>	%
	$\epsilon$	$\gamma^G$	$\gamma^A$	$\psi\beta$	3' $\psi\beta$	5' $\beta$		
Bantu	-	+	-	-	-	-	21	50.0
Benin	-	-	-	-	+	-	15	35.7
Senegal	-	+	-	+	+	+	1	2.4
Atypicals	-	+	-	-	+	-	-	-
	-	-	-	-	-	-	-	-
	-	+	+	-	-	-	5	11.9
Total							42	100.0

<sup>1</sup>Number of chromosomes.

TABLE 2. Intrapopulation haplotype diversity and genetic distance between Sucre-Anzoátegui and other populations based on Beta S haplotype frequencies

Country or population <sup>1</sup>	Genetic distance	Intrapopulation gene diversity
Sucre-Anzoátegui (Venezuela)	—	0.6226
Carabobo-Cojedes (Venezuela)	0.0087	0.6452
México	0.0292	0.6261
Surinam	0.0365	0.6233
Pernambuco (Brazil)	0.0629	0.3246
São Paulo (Brazil)	0.0660	0.4640
Pará (Brazil)	0.0671	0.4680
Bahia (Brazil)	0.0748	0.5070
Cuba	0.0767	0.5683
Mozambique	0.0775	0.6667
St-Tomé-Príncipe	0.0854	0.4224
Various (Venezuela)	0.0901	0.6206
Rio Grande do Sul (Brazil)	0.0921	0.3340
Central African Republic	0.0949	0.2599
Jamaica	0.1066	0.4118
Blacks (USA)	0.1305	0.6124
Guadeloupe	0.1340	0.4472
Angola	0.1681	0.1147
Kenya	0.2155	0.0396
South Africa	0.2161	0.2365
Nigeria	0.2919	0.1516
Tanzania	0.2929	0.0000
Tunisia	0.3405	0.0958
Turkey	0.3548	0.0778
Cameroon	0.3740	0.6092
Algeria	0.4025	0.0000
Benin	0.4025	0.0000
Guinea-Bissau	0.4192	0.8333
Saudi Arabia	0.5782	0.5460
Cape Verde	0.6062	0.3247
Senegal	0.6328	0.2462
Guinea Republic	0.7287	0.0785
India	0.7948	0.2529
Syria	1.000	0.0000

<sup>1</sup>Data obtained from Wainscoat et al., 1983; Antonarakis et al., 1984; Hattori et al., 1986; Nagel et al., 1991; Öner et al., 1992; Zago et al., 1992; Costa et al., 1994; Gonçalves et al., 1994; Muniz et al., 1995; Peñaloza et al., 1995; Kéclard et al., 1996; Olivero, 1996; Franca et al., 1998; Panté de Sousa et al., 1998; Bortolini and Salzano, 1999; Arends et al., 2000.

Haplotype frequencies associated with HBB\*A are shown in Table 3. According to the classification of Long et al. (1990), 11 haplotypes are evident. Haplotypes 2 (39%), 3 (13%), 6 (11%), 1 (9%), 4 (7%), and 5 (7%) are the most frequent, followed by haplotypes 12, 7, 9, 11, and 16 with smaller distributions (4–1%). Atypical haplotypes represent a total frequency of 4%.

Table 4 shows in increasing order the genetic distances estimated from HBB\*A haplotype frequencies between Sucre-Anzoátegui and 17 other populations. The smallest distance (~0.07) is again evident between Sucre-Anzoátegui and another Venezuelan population, Panaquire. Among African populations, the smallest distance is with the Bantu group (0.22) and the largest is with Benin (0.43). The intrapopulation variability of Sucre-Anzoátegui is estimated as 81%.

The corresponding dendrogram based on genetic distances in Table 4 is shown in Figure 3. Sucre-Anzoátegui is located in an intermediate cluster that includes other Venezuelan populations (Panaquire and Maracay) and Blacks from Brazil, who are closely related to another cluster formed by Sub-Saharan Africans (Bantu, Kung, Nigeria, and Benin) and Blacks from the United States. Sucre-Anzoátegui is genetically more distant from Amerindians, Asians, and Europeans.

## DISCUSSION

The Venezuelan gene pool is the product of admixture between Native Americans, European colonizers, and Africans brought as slaves mainly between 1500 and 1810 (Aizpurua, 1988). Venezuelans with an

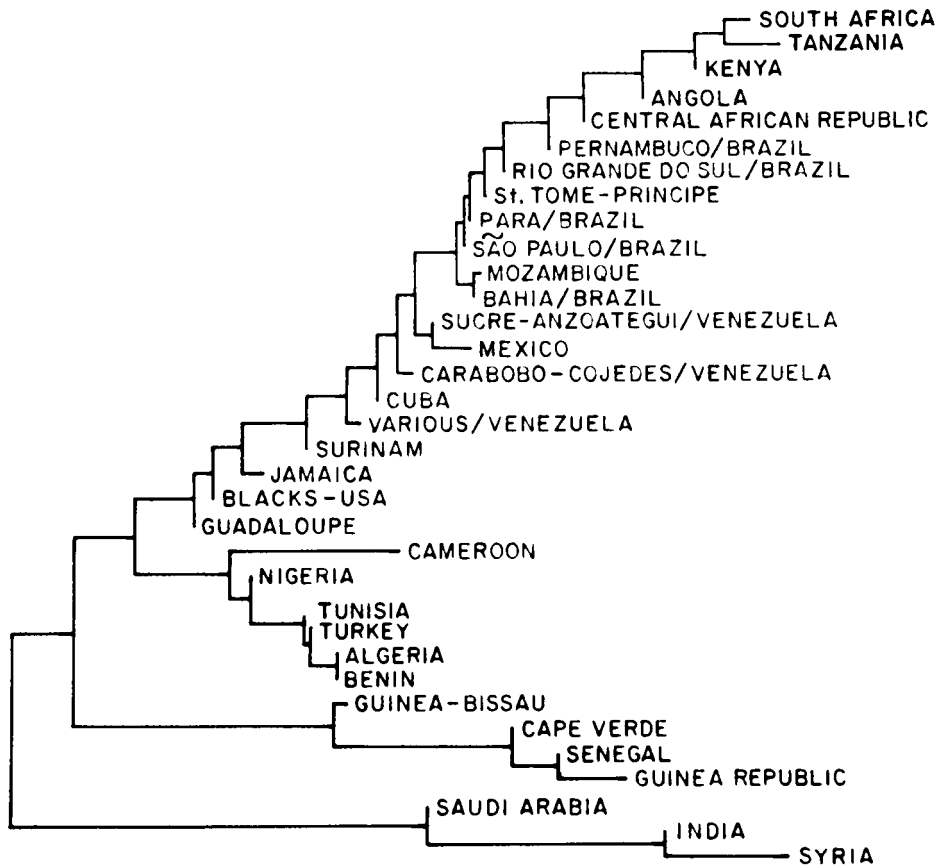


Fig. 2. Dendrogram based on genetic distance between Sucre-Anzoátegui and other populations based on Beta S haplotype frequencies.

important African component occupy a vast region along the Caribbean coast, from the eastern to the western part of the country (Arends, 1971; Brito Figueroa, 1983; Arends et al., 1985, 1990, 2000; Castro de Guerra, 1992; Castro de Guerra et al., 1997).

The presence of high frequencies of Bantu and Benin haplotypes, and in lower frequencies the Senegal haplotype, suggest that the mutation HBB\*S in the Sucre and Anzoátegui states is mainly of Bantu origin. Similar results have been observed in Venezuelan patients with sickle cell anemia from the central states of Carabobo and Cojedes, where the Bantu, Benin, and Senegal haplotype show frequencies of 47%, 36%, and 8%, respectively (Olivero, 1996). On the other hand, the results are not consistent with those published by Arends et al. (2000) for patients from different regions of

Venezuela, where the most common haplotype was Benin (51%), followed by Bantu (32%), Senegal (14%), Cameroon (2%), and atypical haplotypes (0.5%).

Precise historical data about the origin of African slaves brought to Venezuela are non-existent, but some authors trace the origin to the central-western coast of Africa, although it is also reported that some of them were reexported from the Caribbean Islands (Acosta Saignes, 1969; Curtin, 1969). In both regions the most frequent haplotype is Benin. Differences between ours and those of Olivero's (1996) frequencies, and the frequencies reported by Arends et al. (2000), and the available historical data, can be explained by particular conditions related to the origin and development of the African-derived populations in Venezuela, where there are important levels of isolation and endogamy.

TABLE 3. Frequencies of beta A haplotypes in individuals from Sucre-Anzoátegui

Haplotypes <sup>1</sup>	Restriction sites					N <sup>2</sup>	%
	$\epsilon$	$\gamma^G$	$\gamma^A$	$\psi\beta$	$3'\psi\beta$		
1	-	-	-	-	-	7	9.5
2	+	-	-	-	-	29	39.2
3	-	-	-	-	+	10	13.5
4	-	+	-	-	+	5	6.7
5	-	+	-	+	+	5	6.7
6	-	+	+	-	+	8	10.8
7	-	+	+	-	-	1	1.3
9	-	+	+	+	+	1	1.3
11	-	-	-	+	+	1	1.3
12	+	+	-	-	-	3	4.0
16	-	+	-	-	-	1	1.3
Atypical	-	+	-	+	-	3	4.0
	-	-	+	-	+		
Total						74	100.0

<sup>1</sup>According Long et al. (1990).<sup>2</sup>Number of chromosomes.

These factors and correlated phenomena, such as the founder effect and genetic drift, may have favored the increase of the Benin haplotype in some areas and of the Bantu haplotype in others (Castro de Guerra, 1992; Castro de Guerra et al., 1990a,b).

High frequencies of the Benin haplotype have been reported in many other countries of the Americas such as Canada, the United States, Jamaica, Guadeloupe, and Surinam (Antonarakis et al., 1984; Kéclard et al., 1996; Öner et al., 1992). However, in Cuba, Brazil, and Mexico the most frequent haplotype is

Bantu (Muniz et al., 1995; Pante de Souza et al. 1999; Peñaloza et al., 1995). The available data suggest different origins of the HBB\*S allele in the American continent and the occurrence of stochastic events could have favored the presence of the different haplotypes in different areas, increasing intra- and interpopulation variability (Lavinha et al., 1992; Nagel and Ramsay, 1990; Ramsay and Jenkins, 1987).

The level of the intrapopulation haplotype diversity observed in Venezuelan populations is also noted in other American

TABLE 4. Intrapopulation haplotype diversity and genetic distance between Sucre-Anzoátegui and other populations based on beta A haplotype frequencies

Country or population <sup>1</sup>	Genetic distance	Intrapopulation haplotype diversity
Sucre-Anzoátegui (Venezuela)	—	0.8069
Panaquire (Venezuela)	0.0694	0.8070
Algeria	0.1060	0.6767
Blacks (Brazil)	0.1254	0.8176
Blacks (USA)	0.1357	0.8306
Maracay (Venezuela)	0.1968	0.8746
India	0.2023	0.6167
Bantu	0.2218	0.7884
Germany	0.2257	0.7327
Amerindian	0.2260	0.2758
China	0.2361	0.3026
Japan	0.2431	0.2940
Italy	0.2497	0.4974
Korea	0.2587	0.2712
England	0.2829	0.6470
Kung	0.3159	0.7386
Nigeria	0.3162	0.6069
Benin	0.4297	0.6617

<sup>1</sup>Data obtained from Olivero et al., 1996; Castro de Guerra et al., 1997; Guerreiro, 1992; Guerreiro et al., 1994; Panté de Sousa et al., 1999a.

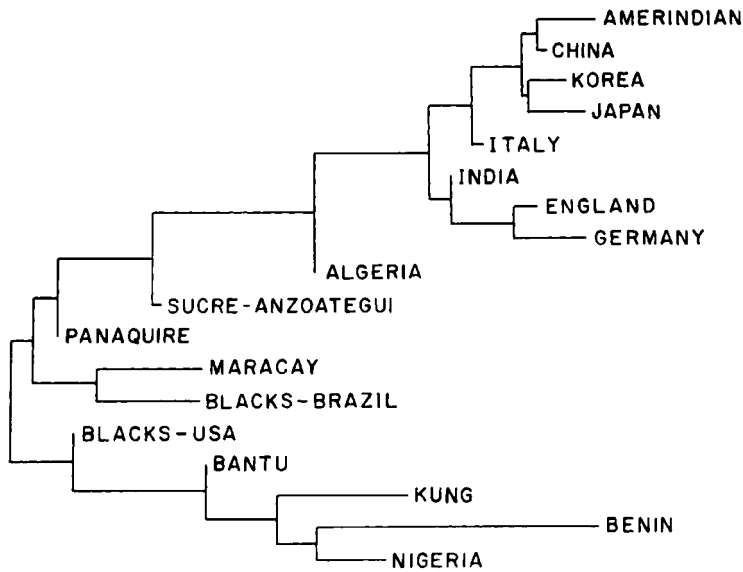


Fig. 3. Dendrogram based on genetic distance between Sucre-Anzoátegui and other populations based on Beta A haplotype frequencies.

countries where sampling was carried out in different areas (Costa et al., 1994; Figueredo et al., 1994; Rodríguez et al., 1994; Wagner et al., 1996). This suggests that sampling is an important factor that needs to be considered.

Genetic distances based on haplotypes of the Beta S gene cluster show that Sucre and Anzoátegui are more related to Bantu and central-south African populations than to those from the central-western coast of Africa, which is consistent with results of studies using classical genetic polymorphisms (Bortolini et al., 1995). However, the level of intrapopulation haplotype variability and the observed frequencies for non-Bantu haplotypes suggest important diversity of the African contingent that arrived in Venezuela.

The allelic disequilibrium observed for haplotypes associated with HBB\*S was also observed for those associated with HBB\*A. In this case, the 11 observed haplotypes represent only 34% of all possible combinations, the most frequent being that identified as 2 (+ - - - -), which is characteristic of non-African groups, followed by 3 (- - - - +), which is characteristic of African groups, and 6 (- + + - +), also of non-African origin. These results indicate

that the level of admixture in this population, as suggested by the elevated level of intrapopulation haplotype diversity (81%), is similar to those observed for other Afro-American groups.

The relationship between populations inferred from genetic distances estimated with HBB\*S haplotypes provides information about the origin of this mutation, but not necessarily about the genetic contribution of Africans or other groups to the gene pool of a particular region. This is best inferred from distances based on HBB\*A haplotypes. In this sense, the high frequency of haplotype 2 in Sucre-Anzoátegui is indicative of the important contribution of non-African groups in the conformation of this population and explains why higher distances than those estimated with HBB\*S were obtained. Tables 2 and 4 show that Sucre-Anzoátegui have the lowest distances with other American populations with similar origins. The large distance between Sucre-Anzoátegui and the Venezuelan population of Maracay in Table 4 can be explained by the high frequency of haplotype 3 in the latter population, which shows lower distances with African groups than Sucre-Anzoátegui (data not shown).

The reduced number of studies published for haplotypes associated with both HBB\*A

and HBB\**S* alleles diminishes the possibility of comparative analysis. However, as observed with HBB\**S*, the genetic distances obtained with HBB\**A* show that the affinity of the population under study with Africans is mainly with Bantu-speaking groups. In addition, both dendrograms show the similarity of Sucre-Anzoátegui with other Afro-American populations.

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