



Beta-globin gene cluster haplotypes in Venezuelan sickle cell patients from the State of Aragua

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Abstract

Seven polymorphic sites in the β -globin gene cluster were analyzed on a sample of 96 chromosomes of Venezuelan sickle cell patients from the State of Aragua. The Benin haplotype was predominant with a frequency of 0.479, followed by the Bantu haplotype (0.406); a minority of cases with other haplotypes was also identified: atypical Bantu A2 (0.042), Senegal (0.031), atypical Bantu A7 (0.021) and Saudi Arabia/Indian (0.021) haplotypes; however, the Cameroon haplotype was not identified in this study. Our results are in agreement with the historical records that establish Sudanese and Bantu origins for the African slaves brought into Venezuela.

Key words: haplotypes, Beta-globin gene cluster, sickle cell.

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Introduction

Sickle cell disease (SCD) exhibits a wide spectrum of clinical behavior: everything from a mild condition to an incapacitating illness; its hematological characteristics, as well as its clinical severity, are influenced by variations in fetal hemoglobin (HbF) levels (Falusi and Olatungi, 1994; Steinberg, 1996), the simultaneous presence of α -thalassemia (Figueiredo *et al.*, 1996; Mukherjee *et al.*, 1997) glucose-6-phosphate dehydrogenase deficiency (Bouanga *et al.*, 1998) and the β -globin cluster linked haplotypes (Powars and Hiti, 1993; Powars *et al.*, 1994; Steinberg, 1996). The β^S gene is in linkage disequilibrium with five main different haplotypes of the β gene cluster, which were named according to the geographical areas where they are most prevalent: Benin, Cameroon, Central African Republic (CAR or Bantu), Senegal and Saudi Arabia/Indian (SAI) (Pagnier *et al.*, 1984; Kulozik *et al.*, 1986; Lapoumériou *et al.*, 1992).

Sickle cell disease was introduced into the American continent mainly by the massive trade of African slaves which occurred between the sixteenth and nineteenth centuries, and is the most common hereditary disorder in the Americas. Sickle cell epidemiological studies carried out in

Venezuela demonstrated a variable frequency, ranging from zero in the Venezuelan Indians up to 5 percent in the Mestizo and Afro-American populations; in some regions such as the North Central Coastal Region the frequency has risen to as much as 12% (Arends, 1971; Arends *et al.*, 1982). In order to provide the required information for further analysis of the heterogeneity of the hematological parameters and clinical manifestations of SCD in Venezuela, the DNA β -globin gene cluster haplotypes were analyzed in 48 Venezuelan sickle cell patients from the State of Aragua. The results were compared with those found in other studies with Venezuelan patients (Arends *et al.*, 2000), Guadeloupe Island (Kéclard *et al.*, 1996) and Iberoamerican populations of Cuba (Muniz *et al.*, 1995), Colombia (Cuellar-Ambrosi *et al.*, 2000) and Brazil (Zago *et al.*, 1992; Figueiredo *et al.*, 1994; Gonçalves *et al.*, 1994; Pante de Sousa *et al.*, 1998, 1999).

Subjects and Methods

The patient sample was composed of 48 non-related HbS homozygotes, from the State of Aragua, which is in the North Central Coastal Region of Venezuela, at about 9°23' to 10°33' N. and 66°33' to 67°53' W. The population of this State is the result of an intense process of admixture among Caucasians, Amerindians, and Afro-Americans and its total population size, according to the 1990 census (OCEI, 1990) represents 6.2% of the Venezuelan

population. These patients were attended at the Hematological Service at the Maracay Central Hospital in the State of Aragua. There were 23 males and 25 females, with a mean age of 21 ± 3.3 years.

Venous blood samples were collected using EDTA as an anticoagulant, and the hematological parameters were determined by standard laboratory procedures. The buffy coat was used for the preparation of nuclear DNA according to previously described methods (Kirby, 1992). Diagnosis of SCD was based on the *Bsu*36I digestion of a PCR amplified 536 bp., verifying whether the β -globin DNA segment contained the β^S mutation, as reported previously (Martínez *et al.*, 1998). Haplotypes were determined by the analysis of the following polymorphic restriction sites in the β -globin gene cluster: 1) *Hinc* II 5' of ϵ ; 2) *Xmn* I 5' of $G\gamma$; 3) *Hind* III in the IVSII of $G\gamma$; 4) *Hind* III in the IVSII of $A\gamma$; 5) *Hinc* II in the $\Psi\beta$; 6) *Hinc* II 3' of $\Psi\beta$; and 7) *Hinf* I 5' β . Segments containing each of these sites were amplified by PCR and subsequently digested with the appropriate enzyme. The oligonucleotide primers used for the analysis of sites 2 to 7 were described by Sutton *et al.* (1989) and site 1 was analyzed using a primer reported by Guerreiro *et al.* (1992).

Results

The β -globin cluster haplotypes identified in the Venezuelan patients are presented in Table I. Benin is the most frequent with a frequency of 0.479, followed by Bantu (0.406), Senegal (0.031), and Saudi Arabia/Indian (0.021). The Bantu A2 and Bantu A7 atypical haplotypes described by Srinivas *et al.* (1988) were observed with a frequency of 0.042 and 0.021, respectively. No Cameroon haplotypes were identified in this study. Fourteen of the 48 SCD patients were homozygous for the Benin haplotype (29.2%), 9 were Bantu homozygotes (18.7%) and all the others were

Table I - β -globin gene cluster haplotypes identified in a sample of 96 chromosomes from Venezuelan sickle cell patients

Haplotype	Number of chromosomes (n = 96)	Frequency
Benin	46	0.479
Bantu	39	0.406
Atypical Bantu A2	4	0.042
Senegal	3	0.031
Saudi Arabia/Indian	2	0.021
Atypical Bantu A7	2	0.021

heterozygotes (52.1%). Table II shows the β^S typical haplotype frequency distribution among the Venezuelan patients studied in this work, compared with those from several Iberoamerican populations and Caribbean Islands.

Discussion

The data reported in this study indicated that the Benin (47.9%) and Bantu (40.6%) haplotypes were the most prevalent, Senegal was present in 3.1% of the patients, a value similar to that found in the western region of Colombia (4.3%) and the Belém population of Brazil (3.4%). The frequencies of the two atypical haplotypes, Bantu A2 (4.2%) and Bantu A7 (2.1%), are some of the highest reported in American populations. This observation can be interpreted as resulting from recombinatorial events involving the Bantu typical haplotype and the 5' regions from various haplotypes present in normal individuals as described by Srinivas *et al.* (1988). In an admixed population such as the Venezuelan, there are more probabilities of observing these events.

Fifty-two percent of the SCD patients studied presented heterozygous haplotypes, as was expected due to the

Table II - Frequency distribution (%) of the typical β^S haplotypes in patients from Iberoamerican populations and the Guadeloupe Island.

Population	N. of chromosomes	Haplotypes				
		Bantu	Benin	Senegal	Arab/Indian	Cameroon
Venezuela (State Aragua) ¹	90	43.3	51.1	3.3	2.2	0.0
Venezuela (All country) ²	176	32.4	51.1	14.2	0.0	2.3
Cuba ³	198	41.0	51.0	8.0	0.0	0.0
Colombia (Western Region) ⁴	92	55.5	34.8	4.3	0.0	5.4
Brazil (Afro-Brazilians from the Amazon Region) ⁵	20	60.0	10.0	30.0	0.0	0.0
Brazil (Belém, State of Pará) ^{6,7}	59	66.1	30.5	3.4	0.0	0.0
Brazil (Salvador, State of Bahia) ⁷	42	55.0	45.0	0.0	0.0	0.0
Brazil (Ribeirão Preto, State of São Paulo) ^{7,8}	67	73.0	25.5	1.5	0.0	0.0
Brazil (Campinas, São Paulo) ⁹	142	79.6	18.4	2.0	0.0	0.0
Guadeloupe ¹⁰	252	11.5	77.0	8.7	0.0	2.8

¹Present study; ²Arends *et al.* (2002); ³Muniz *et al.* (1995); ⁴Cuellar-Ambrosi *et al.* (2000); ⁵Pante de Sousa *et al.* (1999); ⁶Pante de Sousa *et al.* (1998); ⁷Figueiredo *et al.* (1994); ⁸Zago *et al.* (1992); ⁹Gonçalves *et al.* (1994). ¹⁰Kéclard *et al.* (1996).

admixture process which occurs in our population; in a study carried out on African SCD patients, the vast majority of haplotypes were found in homozygosity (Sow *et al.*, 1995). Our results differed significantly from those reported by Arends *et al.* (2000) in another study performed on sickle cell Venezuelan patients from all over the country ($\chi^2 = 14.62$; d.f. = 4; $p < 0.01$). This may be due to different patient ascertainment and/or a diverse historical origin of the African-derived patients from Aragua. On the other hand, our results are in agreement with the haplotype distribution found in patients from Cuba ($\chi^2 = 6.60$; d.f. = 3; $p > 0.05$); while significant differences exist with the Colombian data ($\chi^2 = 11.23$; d.f. = 4; $p < 0.025$). A highly significant difference was observed when the Venezuelan patients were compared with those from Guadeloupe ($\chi^2 = 42.63$ d.f. = 2; $p < 0.001$), and those from Brazil ($\chi^2 = 20.03$; d.f. = 3; $p < 0.001$). It is probable that all these differences reflect the origin of the forced migration of African slaves to the American continent, although, as was indicated for the two Venezuelan samples, patient ascertainment should also be considered.

African slavery was instituted in Venezuela to meet the growing labor demands of an emerging agricultural economy; historically it is reported that the African slaves brought to Venezuela were from the Sudan and Bantu regions (Acosta Saignes, 1984), although some slaves came to Venezuela from the others colonies, especially the Antilles; in consequence, the predominance of the Benin and Bantu haplotypes is in accordance with these historical records.

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