



Short Communication

Determination of β^S haplotypes in patients with sickle-cell anemia in the state of Rio Grande do Norte, Brazil

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Abstract

β^S haplotypes were studied in 47 non-related patients with sickle-cell anemia from the state of Rio Grande do Norte, Brazil. Molecular analysis was conducted by PCR/RFLP using restriction endonucleases XmnI, HindIII, HincII and HinfI to analyze six polymorphic sites from the beta cluster. Twenty-seven patients (57.5%) were identified with genotype CAR/CAR, 9 (19.1%) CAR/BEN, 6 (12.8%) CAR/CAM, 1 (2.1%) BEN/BEN, 2 (4.3%) CAR/Atp, 1 (2.1%) BEN/Atp and 1 (2.1%) with genotype Atp/Atp. The greater frequency of Cameroon haplotypes compared to other Brazilian states suggests the existence of a peculiarity of African origin in the state of Rio Grande do Norte.

Key words: haplotypes, β -globin, sickle-cell anemia, Brazilian population, S hemoglobin.

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Sickle-cell anemia, which results from the homozygosis of β^S allele [beta 6(A3) Glu > Val] of beta globin gene, is the most common and most serious form of sickle-cell disease (Serjeant, 1997).

β -globin gene haplotype is characterized by the non-random association of cleavage sites recognized by restriction endonucleases (Steinberg *et al.*, 1996). The main β^S haplotypes regularly found in patients with sickle-cell anemia, worldwide, are named according to the geographic region or ethnic group in which they were originally identified, *i.e.*, Senegal (SEN), Benin (BEN), Bantu or Central African Republic (CAR), Cameroon (CAM) and Arab-Indian (ARAB), (Powars, 1991). Other less common haplotypes, known as atypical haplotypes (Atp), are generated by a number of genetic mechanisms (Zago *et al.*, 2000).

The analysis of β^S haplotypes, besides being an important source for anthropological studies on the ethnic

composition of a population, contributes to a better understanding of the variation in clinical seriousness of sickle-cell anemia, given that the different types of haplotypes contain a range of fetal hemoglobin concentrations that act as inhibitors of hemoglobin S polymer formation (Flint *et al.*, 1998; Inati *et al.*, 2003).

Senegal and Arab-Indian haplotypes, by producing the highest levels of fetal hemoglobin in the blood, are associated with less severe clinical evolution of sickle-cell anemia, with a lower occurrence of organic damage. As to the Benin and Cameroon haplotypes, the clinical picture is of intermediate severity. The Bantu or CAR haplotype is associated with greater clinical severity (Powars, 1991; Zago *et al.*, 1992; Bortolini and Salzano, 1999).

In light of the absence of studies on haplotypes in the state of Rio Grande do Norte, the aim of the present investigation was to determine β^S haplotypes in a group of patients with sickle-cell anemia.

A total of 47 non-related individuals with sickle-cell anemia (25 males and 22 females) from the state of Rio Grande do Norte, aged between 10 months and 46 years (mean 16.1 ± 10.4 years), were analyzed. These patients

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were enrolled at the Hematology Ambulatory Care Facility of the Dalton Barbosa Cunha Hemocenter, Natal, RN, Brazil and the Oncology and Hematology Center of Mossoró, RN, Brazil. The research project was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (CEP-UFRN, protocol number 090/07). All the patients or their legal guardians gave written informed-consent after being instructed on the aim of the study and the procedures involved.

Samples of venous blood were collected using EDTA as anticoagulant. The hemoglobin profile was determined by cellulose acetate electrophoresis at alkaline pH (Dacie and Lewis, 1995), and the presence of HbS confirmed by means of the solubility test (Magalhães and Arashiro, 1977). HbA₂ was measured by elution from cellulose acetate strips following electrophoresis (Bezerra, 1984). The level of HbA₂ was used to exclude the interaction S-β thalassemia. DNA was isolated from peripheral blood leukocytes using the illustra blood genomicPrep Mini Spin kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK). β^S haplotypes were investigated by PCR/RFLP. PCR was performed using primers H0, H1, H2, H3, H4, H5, H6, H7, H8, H9 and H10 (Sutton *et al.*, 1989) in a GeneAmp 9700 thermocycler (Applied Biosystem, Foster City, CA, USA). The analysis of polymorphic regions was by incubating PCR products with the restriction endonucleases *Xmn*I, *Hind*III, *Hinc*II and *Hinf*I, for 12 h at 37 °C. Amplified products were visualized and polymorphic sites identified using 2% agarose gel electrophoresis, followed by ethidium bromide staining and photodocumentation (Sambrook *et al.*, 1989). A 100 bp DNA Ladder (BioLabs) was applied in each gel electrophoresis as a standard molecular weight marker. Pre-determined homozygous and heterozygous patterns were used for each haplotype investigated.

The samples were marked by the presence (+) or absence (-) of each of the 6 restriction sites analyzed in the beta-globin gene cluster (*Xmn*I 5'γ^{G0}, *Hind*III in γ^G and γ^A, *Hinc*II in 3'ψβ and ψβ, and *Hinf*I in 5'β). According to the combination of results, the five most common β^S haplotypes were defined as follows: CAR (- + - - -), Benin (- - - + -), Senegal (+ + - + +), Arab-Indian (+ + - + +) and Cameroon (- + + - +).

Of the 94 chromosomes analyzed, 71 (75.5%) were characterized as CAR, 12 (12.8%) as Benin, 6 (6.4%) as Cameroon and 5 (5.3%) as atypical haplotypes.

In relation to the combination of β^S haplotypes, 27 (57.5%) patients carried the CAR/CAR genotype, 9 (19.1%) the CAR/BEN, 6 (12.8%) the CAR/CAM, 1 (2.1%) the BEN/BEN, 2 (4.3%) the CAR/Atp, 1 (2.1%) the BEN/Atp, and 1 (2.1%) the Atp/Atp.

The frequency of β^S haplotypes varies according to origin. The African presence in Brazil began around 1550, as a replacement for native labor. The main ports of entry were Salvador, Recife and Rio de Janeiro. Individuals from

Nigeria, Daomé (present Day Benin) and the Ivory Coast were sent mainly to Bahia, whereas the Bantus, captured in the Congo, Angola and Mozambique, were sent to Pernambuco, Minas Gerais and Rio de Janeiro (Vicentino and Dorigo, 2002).

Since there were no ports or slave trade, the state of Rio Grande do Norte was not greatly influenced by African origin in its ethnic formation of the population. Moreover, unlike other regions, there was no economic activity requiring slave labor. As a result, only a small number of slaves, mainly from Pernambuco, were sent to this state (Casculo, 1955).

Frequency distribution of β^S haplotypes in the patients included in this study and in others involving various Brazilian states, appear in Table 1. A general predominance of the CAR haplotype can be observed in the country (Zago *et al.*, 1992; Gonçalves *et al.*, 1994; Pante de Souza *et al.*, 1998; Cardoso and Guerreiro, 2006; Bezerra *et al.*, 2007; Silva *et al.*, 2009). The Benin haplotype is present in all regions, with high frequency in Bahia (Costa *et al.*, 1984; Gonçalves *et al.*, 2003; Adorno *et al.*, 2004) and Rio de Janeiro (Fleury, 2007).

The high frequency of CAR haplotype (75.5%) found in the present study, followed by the Benin haplotype (12.8%), was comparable to that obtained by Bezerra *et al.* (2007) in Pernambuco, the main source of slaves for Rio Grande do Norte. The frequency found for the Cameroon haplotype (6.4%) was higher than that observed in other states such as Bahia, and even Pernambuco (Adorno *et al.*, 2004, 2008; Cardoso and Guerreiro, 2006; Bezerra *et al.*, 2007). Statistical analysis using the proportion comparison test showed a significant difference, except for the study conducted by Adorno *et al.* (2004) ($p = 0.0943$). No Senegal or Saudi Arabian haplotypes were identified in the study sample.

The record of a shipment of 2,400 African slaves from Andra, Benin, Warri (Niger Delta), Calabar and Cameroon to Pernambuco in 1638, during the Dutch occupation, confirms the entry of Africans from a region with high Cameroon haplotype frequency into northeastern Brazil (Alencastro, 2000). After 1845, with the rise in sugarcane production, slavery in Rio Grande do Norte increased, bolstered by the purchase of slaves from other states, such as Pernambuco and Maranhão (Medeiros, 1978). Hence, a possible explanation of the high frequency found in the present study, albeit also attributable to the small number of patients analyzed herein.

The present study constitutes the first attempt to investigate the frequency of β^S haplotypes in the population of the state of Rio Grande do Norte. The higher frequency of the Cameroon haplotype, compared to other Brazilian states, suggests the existence of a peculiarity of African origin.

Table 1 - Summary of studies conducted in Brazil to investigate β^S haplotypes.

Author/year	Population	Nº of patients	Haplotypes β ^S (%)					
			CAR	BEN	CAM	SEN	ARAB	Atp
Costa <i>et al.</i> (1984)	São Paulo (SP)	37	61.0	38.0	-	-	-	1.0
Zago <i>et al.</i> (1992)	São Paulo (SP)	37	66.2	23.0	-	1.3	-	9.5
Gonçalves <i>et al.</i> (1994)	São Paulo (SP)	74	62.2	33.8	-	-	-	4.0
Fleury (2007)	Rio de Janeiro (RJ)	74	54.0	44.6	-	1.4	-	-
Costa <i>et al.</i> (1984)	Salvador (BA)	36	49.0	51.0	-	-	-	-
Gonçalves <i>et al.</i> (2003)	Salvador (BA)	80	48.1	45.6	-	0.7	-	5.6
Adorno <i>et al.</i> (2004)	Salvador (BA)	80	46.2	48.8	1.9	0.6	-	0.6
Adorno <i>et al.</i> (2008)	Salvador (BA)	125	41.6	55.2	1.2	0.4	0.4	1.2
Bezerra <i>et al.</i> (2007)	Recife (PE)	74	81.1	14.2	0.8	-	-	3.9
This study	Natal (RN)	47	75.5	12.8	6.4	-	-	5.3
Galiza Neto <i>et al.</i> (2005)	Fortaleza (CE)	22	31.8	43.2	-	2.3	-	-
Silva <i>et al.</i> (2009)	Fortaleza (CE)	34	66.2	22.0	-	-	-	11.8
Pante de Sousa <i>et al.</i> (1998)	Belém (PA)	30	67.0	30.0	-	3.0	-	-
Cardoso and Guerreiro (2006)	Belém (PA)	130	66.0	21.8	1.3	10.9	-	-

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