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Sequence change in the HS2-LCR and Gγ-globin gene promoter region of sickle cell anemia patients

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The fetal hemoglobin (HbF) levels and ß^S-globin gene haplotypes of 125 sickle cell anemia patients from Brazil were investigated. We sequenced the Gγ- and Aγ-globin gene promoters and the DNase I-2 hypersensitive sites in the locus control regions (HS2-LCR) of patients with HbF level disparities as compared to their ß^S haplotypes. Sixty-four (51.2%) patients had CAR/Ben genotype; 36 (28.8%) Ben/Ben; 18 (14.4%) CAR/CAR; 2 (1.6%) CAR/Atypical; 2 (1.6%) Ben/Cam; 1 (0.8%) CAR/Cam; 1 (0.8%) CAR/Arab-Indian, and 1 (0.8%) Sen/Atypical. The HS2-LCR sequence analyses demonstrated a c.-10.677G>A change in patients with the Ben haplotype and high HbF levels. The Gγ gene promoter sequence analyses showed a c.-157T>C substitution shared by all patients, and a c.-222_-225del related to the Cam haplotype. These results identify new polymorphisms in the HS2-LCR and Gγ-globin gene promoter. Further studies are required to determine the correlation between HbF synthesis and the clinical profile of sickle cell anemia patients.

Key words: Fetal hemoglobin; Sickle cell anemia; ß^S-globin gene haplotypes; Locus control region; γ-globin promoter

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Patients with sickle cell anemia (SCA) have a wide spectrum of clinical manifestations, characterized by heterogeneous severity, that are frequently associated with the levels of fetal hemoglobin (HbF) (1). The ß^S-globin gene haplotypes are related to polymorphic restriction enzyme sites distributed throughout the ß^S-globin gene cluster region, and have been associated with specific HbF levels and the phenotypic diversity presented by SCA patients (1,2). The major ß^S-globin gene haplotypes have been named Benin (Ben), Central African Republic (CAR), Senegal (Sen), Arab-Indian, and Cameroon (Cam), according to their geographical origin and the ethnic groups in which they are frequently found (2,3).

Previous reports have investigated the roles of ß^S haplotypes and HbF levels as markers of clinical heterogeneity in SCA (2-4). For example, there is a T>A variation in the Aγ-globin gene promoter region (-499) that is associated with the Ben haplotype among Sicilian and North American individuals (5). Most patients with the CAR haplotype have very low HbF levels, often below 5% of total HbF, while Ben haplotype carriers have intermediate HbF levels, from 5 to 15% (4).

Hemoglobin gene switching depends on complex interactions of stage-specific transcription factors, chromo-

somal gene order, proximity to the globin locus control region (LCR), and erythroid-specific and ubiquitous trans-acting factors that interact with the promoter regions (3,6). Some mutations in the γ-globin gene promoter region are associated with increased HbF synthesis, such as the c.-158 C>T polymorphism has been described in the Sen haplotype (2,3). The LCR is characterized by a series of five DNase I-hypersensitive sites (5'-HS1-HS5). Of particular importance is the 5'-HS2-LCR enhancer, which contains a 46-base pair (bp) binding sequence for the NF-E2 and AP-1 trans-acting factors. The 5'-HS2-LCR is flanked by multiple *cis*-acting sequences that modulate enhancer activity, that contain different number of AT-rich sequences and some single-nucleotide polymorphisms (7,8).

Here, we suggest that sequence variations in both the promoter regions for the Gγ- and Aγ-globin genes and the HS2-LCR regions may be important in establishing the HbF level diversity found among specific ß^S-globin gene haplotypes in SCA patients from Northeastern Brazilian (9,10).

The ß^S haplotypes and HbF levels of a group of 125 SCA patients from the Blood Center of Bahia, Salvador, BA, Brazil (HEMOBA) were investigated. The study was approved by the Oswaldo Cruz Research Foundation's human research Ethics Committee. The ß^S haplotypes were identified using polymerase chain reaction and restriction fragment length polymorphism techniques (11). The HbF levels were estimated using high-performance liquid chromatography (VARIANT I, BIO-RAD, Hercules, CA, USA). The Gγ- and Aγ-globin gene promoters and HS2-LCR regions (6,7,12) were sequenced in an ABI Prism 3100 DNA Sequencer using Kit BigDye 03 Terminator™ Sequencing Standards (Applied Biosystems, Foster City, CA, USA) and specific sequencing primers (13).

The frequencies of various ß^S haplotypes in the 125 patients were 64 (51.2%) with genotype CAR/Ben; 36 (28.8%) Ben/Ben; 18 (14.4%) CAR/CAR; 2 (1.6%) CAR/Atypical; 2 (1.6%) Ben/Cam; 1 (0.8%) CAR/Cam; 1 (0.8%) CAR/Arab-Indian, and 1 (0.8%) Sen/Atypical. Among these, 4 CAR/CAR patients had HbF levels ≥10.0%, 3 Ben/Ben patients had HbF ≤5.0%, 11 CAR/Ben patients had HbF ≥15.0%, 18 CAR/Ben patients had HbF ≤5.0%, and 2 Cam/Ben patients had HbF ≥15.0%. From this sample group, ten patients presenting a disparity between the level of HbF and the ß^S haplotype were selected for sequencing of the Gγ- and Aγ-globin gene promoter and HS2-LCR regions. Two Cam/Ben patients were selected, with HbF levels of 17.1 and 27.4%. Two Ben/Ben patients were selected, with HbF levels of 11.5 and 1.3%. Three CAR/Ben patients were selected, with HbF levels of 1.2, 10.1, and 17.6%. Two CAR/CAR patients were selected, with HbF levels of 8.7 and 14.9%. The CAR/Atypical patient was also studied and had an HbF level of 9.6%. The HbF levels and clinical characteristics of the ten patients are shown in Table 1.

Sequencing of the Gγ- and Aγ-globin gene promoter regions confirmed the presence of specific polymorphisms (14), including c.-533_-532*inv* (AGA to AAG) and c.-400_-395del in the 5' region of the Gγ gene, and c.-225_-222del in the 5' region of the Aγ gene. We also found a nucleotide

Table 1. Summary of fetal hemoglobin levels (HbF), age, ß^S-globin gene haplotype and polymorphic *cis*-acting sequences found among sickle cell anemia patients from Northeast Brazil.

Patient	HbF (%)	Age (years)	Complications	Red blood cell transfusions	ß^S haplotype	HS2-LCR	Gγ	
						-10,677	-225 -222	-157
1	27.4%	2	pneumonia, spleen sequestration, painful crisis	No	Cam/Ben	G/A*	N/d*	C/C*
2	17.1%	3	painful crisis	No	Cam/Ben	G/A*	N/d*	C/C*
3	11.5%	45	leg ulcer, painful crisis	Yes	Ben/Ben	G/A*	N/N	C/C*
4	1.3%	21	leg ulcer	Yes	Ben/Ben	G/G	N/N	C/C*
5	1.2%	24	pneumonia, painful crisis	Yes	CAR/Ben	G/G	N/N	C/C*
6	10.1%	7	cerebrovascular accident	Yes	CAR/Ben	G/A*	N/N	C/C*
7	17.6%	10	pneumonia, spleen sequestration	No	CAR/Ben	G/A*	N/N	C/C*
8	8.7%	3	spleen sequestration	Yes	CAR/CAR	G/G	N/N	C/C*
9	14.9%	26	-	Yes	CAR/CAR	G/G	N/N	C/C*
10	9.6%	27	painful crisis	Yes	CAR/Atypical	G/G	N/N	C/C*

Cam = Cameroon; Ben = Benin; CAR = Central African Republic. In Dnase I-2 hypersensitive sites in the locus control regions (HS2-LCR), G/A* = a G to A substitution and G/G = reference nucleotide in the -10,677 position. In the Gγ-globin gene promoter, d = 4-bp deletion between -225 and -222 position, N = reference sequence; C/C* = a T to C substitution in the -157 position. *Sequence variations described in patients investigated in the present study.

substitution at c.-157T>C detected in the Gγ-globin gene promoter (GenBank accession No. bankit 818161, DQ873521; Figure 1), suggesting that this polymorphism, located in the vicinity of another polymorphism (c.-158C>T), is associated with high γ-globin gene expression (2,3). To confirm this finding, it is necessary to know its distribution in other Afro-descendant populations from Bahia, however.

The Ben/Cam patients presented the c.-222_-225del of the Aγ-globin gene promoter region, which may be part of a *cis*-acting element that increases the expression of γ-globin gene when bound to a specific *trans*-acting factor (15). Furthermore, the two patients with the Cam haplotype had high HbF levels, and polymerase chain reaction amplification with primers that were specific and distinct for the

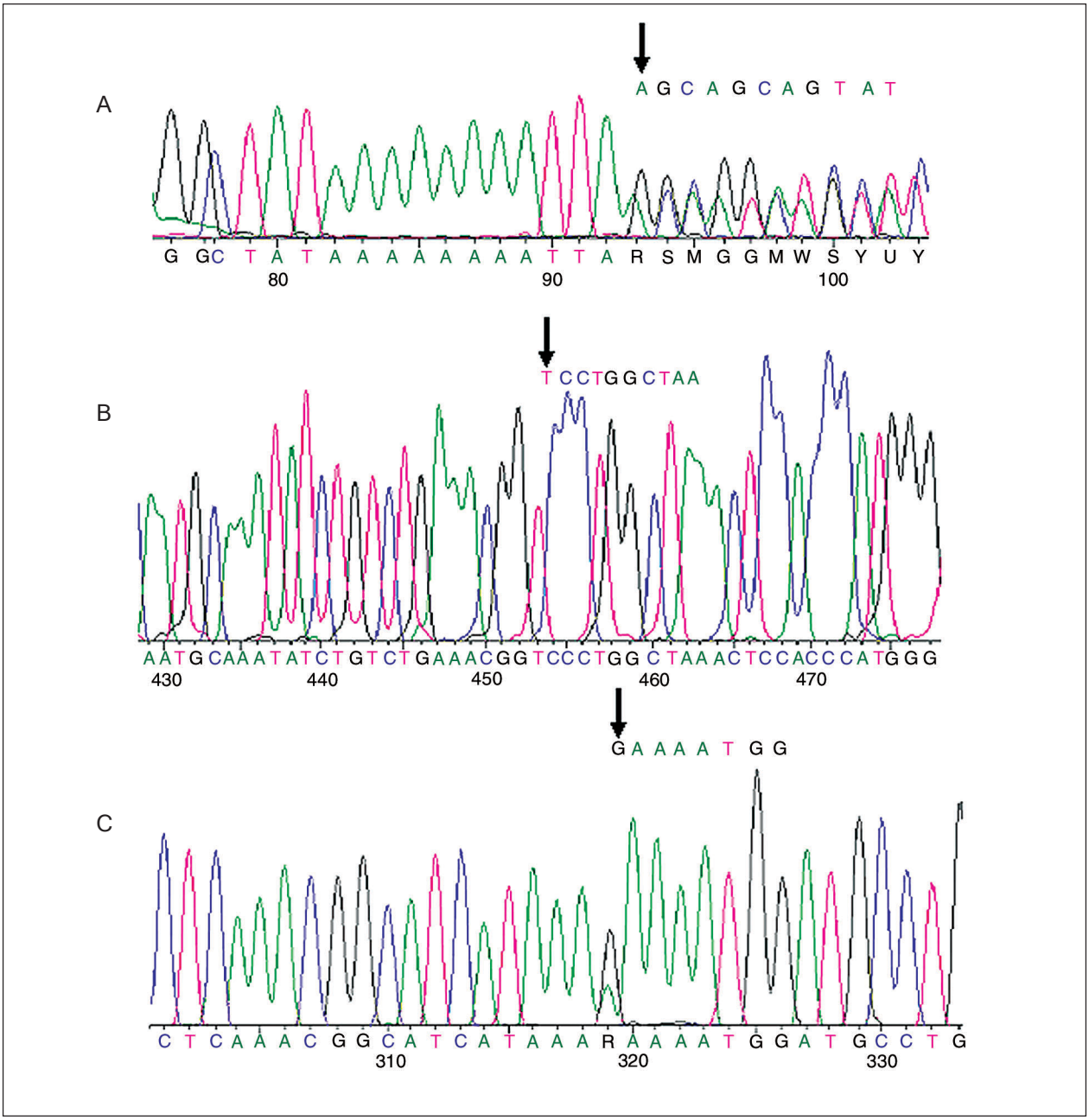

Figure 1. Sequence analysis. *A*, c.-222_-225del of the Gγ-globin gene promoter region described in the Cam haplotype. *B*, Nucleotide substitution (c.-157T>C) at the Gγ-globin gene promoter found in all patients. *C*, Nucleotide substitution (c.-10.677G>A) outside the core sequences of HS2-LCR found in the Ben haplotype.

promoter regions of either the Gγ- or Aγ-globin genes demonstrated a similar c.-222_-225del of the Gγ-globin gene (GenBank accession No. bankit 825906, DQ873519; Figure 1).

We detected a G>A substitution outside the core sequences of HS2-LCR (-10.677 position; GenBank accession No. bankit 825964, DQ873522) in 5 SCA patients with the Ben haplotype and high HbF levels (Figure 1). The CAR/Ben and Ben/Ben patients with low HbF levels did not have this sequence variation. In the small patient group investigated, there were two CAR/CAR patients with high HbF levels without this G>A substitution (Table 1), suggesting that this substitution plays an important role in the regulation of γ-globin gene expression when associated with the Ben haplotype, probably by affecting interaction with a binding site for a specific *trans*-acting factor. This HS2-LCR sequence change is located close to a binding site for GATA-1 and a ubiquitous *trans*-acting factor, and can constitute a motif associated with the Ben chromosome (2-4). The other HS2-LCR polymorphisms described here have been related to specific haplotypes in agreement with previous studies (7,8).

This study confirms the relationship between genotypic heterogeneity and HbF levels in Brazilian SCA patients, emphasizing the high racial admixture of the Bahian Africans population that came from the slave trade from Western Africa, mainly from the Bay of Benin (16). Further studies need to be conducted in the same population in order to clarify the role of new biological markers in the clinical progress of SCA patients, however. The sequence analyses presented herein have identified a new polymorphic site on the promoter region for the Gγ-globin gene, suggestive of a common sequence characteristic among Brazilian SCA patients. The new sequence variation in the HS2-LCR region may be associated with γ-globin gene expression.

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