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DNAase I hypersensitive site 3' to the β-globin gene cluster contains a TAA insertion specific for β^S-Benin haplotype

SILVANA BORDIN,* VANESSA G. CRESPI,* ADRIANA S.S. DUARTE,* DANIELA S. BASSÈRES,* MÔNICA B. MELO,* ALEXANDRA P. ZILLI VIEIRA,* SARA T.O. SAAD,*° FERNANDO F. COSTA*°

*Centro de Hematologia e Hemoterapia, °Departamento de Clínica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 13083-970, Campinas, São Paulo, Brasil

Background and Objectives. Analysis of DNA polymorphic sites is a powerful tool for detection of gene flow in human evolutionary studies and to trace genetic background associated with abnormal genes. The β-globin locus contains more than 20 single-base restriction fragment length polymorphism (RFLP) sites spanning over 80 kb on chromosome 11. Far downstream of the expressed genes, there is a hypersensitive site (HS). The function of the 3'-HS remains unknown. As an approach to the understanding of the 3'-HS region in sickle cell anemia we searched for sequence polymorphism in the AT-rich region, using a non-radioactive polymerase chain reaction (PCR)-single strand conformational polymorphism (SSCP) technique.

Design and Methods. A 460 bp fragment located at the 3' of the β globin gene was amplified from patients (with sickle cell anemia and HbSC disease), and from AS individuals. Standard RFLP-haplotyping was performed and compared with the PCR-SSCP screening strategy.

Results. Two distinct band patterns were revealed by SSCP testing, each one in strict linkage disequilibrium with either Benin or Bantu haplotypes. Direct sequencing of the amplified segment revealed a TAA insertion in the AT-rich region, in all 121 β^S Benin chromosomes tested, but not in other β^S haplotypes from the total of 380 β^S chromosomes typed.

Interpretation and Conclusions. SSCP analysis could easily distinguish sequence variations in the 3'AT-rich region of the β-globin cluster, and a TAA insertion in this region seems to be specific for the Benin-β^S chromosome.
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Key words: sickle cell anemia, β-globin haplotypes, simple sequence repeats, LCR, polymorphism, human

Correspondence: Fernando F. Costa, MD, Hemocentro-UNICAMP, C.P. 6198, 13083-970 Campinas São Paulo, Brazil.
Fax: international +55.19.32891089. E-mail: ferreira@unicamp.br

The β-globin locus, one of the most extensively studied of all human loci, contains over 20 single-base restriction fragment length polymorphism (RFLP) sites, located throughout 80kb on chromosome 11. Early molecular studies of DNA polymorphisms linked to the β-globin gene cluster have demonstrated that a set of RFLPs is not randomly distributed in a given population. This finding has permitted the origin and spread of some of the most common genetic disorders, such as sickle cell anemia (SCA) and β-thalassemia, to be traced worldwide. RFLP-haplotype analyses have indicated, for example, that β^S gene mutations have occurred independently several times in Africa in geographically separated regions, whereas the β^C mutation probably resulted from a unique chromosomal background.[1]

More recently, additional haplotype-linked polymorphic simple sequence repeats have been identified along the β-globin gene cluster.[2] Of particular importance is a segment of contiguous alternating pairs of purine-pyrimidine bases in a subdomain of the 5'-DNAaseI hypersensitive site 2 (HS2), lying in an enhancer element 5' of the ε-globin gene denominated locus control region (LCR). The β-globin LCR exerts a dominant function in the transcription of developmentally active globin genes, in the opening of chromatin structure, and in replication timing of the entire locus.[3]

As haplotype-specific nucleotide variations within simple sequence repeat polymorphisms of the 5'HS2-LCR are located in regulatory regions of the β-globin gene cluster, it has been proposed that these markers may not be neutral on the expression of the β-like globin genes. A microsatellite-like AT-rich region is also found in the 3'-DNAase I hypersensitive site (3'-HS) located 20 kb downstream to the β globin gene.[4] The 3'HS ele-

ment constitutes a nuclear scaffold associated region and, like the 5'-LCR, is involved in chromosomal organization. In spite of the functional importance of the 3'-HS region, to the best of our knowledge, structural alterations in this region have not been investigated.

As an approach to investigate structural variations in 3'-HS, we searched for sequence polymorphisms in the AT-rich region, and identified a TAA insertion in this region, apparently restricted to the Benin haplotype.

Design and Methods

Source and preparation of DNA

This study included 108 patients with sickle cell anemia (SCA), 28 with HbSC disease, and 157 individuals with sickle cell trait (AS) identified during routine screening for hemoglobinopathies at the Center of Hematology and Hemotherapy at the State University of Campinas (UNICAMP).

PCR amplification and single strand conformational polymorphism (SSCP) analysis

To determine the sequence configuration of the AT-rich region of the 3'-HS element, a 460 bp fragment was amplified using PCR primers designed from the reference sequence (Genbank file HSDNA11, coordinates 1995 to 2455). The location of this region and the PCR primers used are shown in Figure 1. Genomic DNA was obtained from patients (with SCA or HbSC disease), from normal blood donors and from individuals with sickle cell trait identified during routine screening for hemoglobinopathies in our Center at UNICAMP. RFLP-haplotyping was performed by amplification of six β-cluster segments containing the following restriction site polymorphisms: Hind III in IVS-2 of $^G\gamma$ and $^A\gamma$, Hinc II in psi ψβ and 3' to it, Hinf I 5' to β, Hpa I 3' to the β gene. Partial haplotype analysis of the SCA patients has been previously reported.[5] Non-radioactive SSCP analysis of the amplified fragment was carried out using an automated apparatus (Phast-System, Pharmacia, Sweden) as previously reported.[6] Running conditions were 200Vh at 10°C. PCR products were further sequenced using a Thermosequenase cycle sequencing kit as recommended by the supplier (Amersham Life Science, UK).

Results

In all SCA patients the RFLP-haplotypes had been previously characterized. SSCP analysis showed two distinct band patterns, each in strict linkage disequilibrium with either Benin or Bantu haplotypes. Direct sequencing analysis of several samples identified a sequence variation linked to the Benin haplotype, whereas the Bantu haplotype was identical to the reference sequence. One Senegal and one Arab-Indian β^S chromosome also carried the 3'-HS reference sequence (Figure 1). The Benin-linked polymorphism consisted of the insertion of the trinucleotide TAA in the TA-rich region (Genbank coordinate 2034).

We further analyzed the presence of this polymorphism in 28 patients with SC disease and in 157 individuals with HbAS. Simultaneously, RFLP-haplotyping was carried out in SC and AS samples in order to identify β^S-Benin and β^S-Bantu alleles. β^C-bearing chromosomes showed the same SSCP band pattern as that found in the β^S-Bantu and

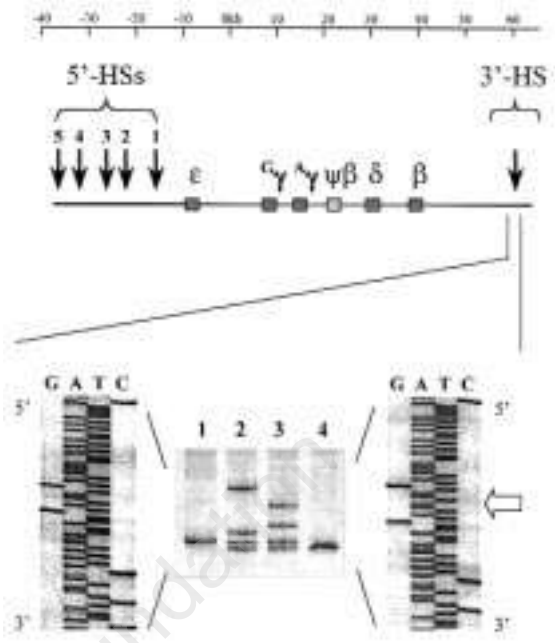

Figure 1. Schematic representation of the β-globin gene cluster. At the top is the map of the β-globin gene cluster below which the amplified segment of 3'-HS is expanded. Arrows indicate DNAase I hypersensitive sites. Sequences and location of the PCR primers used are shown below. The expanded view of the 3'-HS shows the sequencing of reference (left) and β^S-Benin (right) AT-rich regions. Polymorphic patterns detected by PCR-SSCP (center) are obtained from samples 1, β^S Bantu homozygous; 2, β^S Benin/Bantu heterozygous; 3, normal individual (atypical pattern); and 4, β^S Benin homozygous. Sense primer 5'-GGC-TAC-AGT-TGA-ACA-GAT-GGA-CCA (Genbank coordinate 1995. Antisense primer 5'-TTA-CCA-CAA-ACC-TGA-AGT-AGG-C (Genbank 2455).

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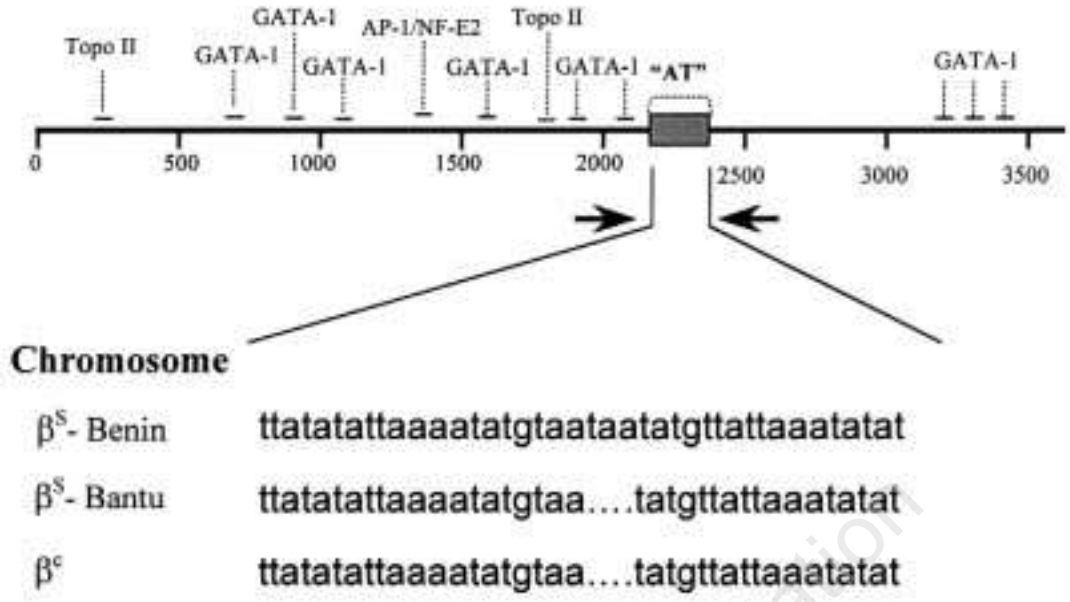

Figure 2. Sequence comparison of the 3'-HS region between Benin and Bantu β^S, and β^C chromosomes. The diagram on top, based on previously published data,[4] shows the arrangement of putative nuclear factor binding motifs. The box denoted by "AT" represents the AT-rich region studied. Horizontal arrows indicate PCR primers.

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reference chromosomes.

Among all β^S chromosomes completely typed, all β^S-Benin have the novel TAA allele (121 out of 121 β^S-Benin chromosomes). All β^S-BANTU and β^C chromosomes were identical to the reference chromosome (Figure 2). Thirteen percent of AS samples could not be resolved because they exhibited a heterozygous pattern in the restriction analysis.

Discussion

Using SSCP analysis of PCR-amplified DNA we were able to identify a new DNA polymorphism in the distal 3 of the β-globin gene cluster. This polymorphism consisted of the insertion of the trinucleotide TAA in the TA-rich region and seems to be linked to the Benin haplotype. It should be pointed out that β^S-Benin and β^S-Bantu haplotypes comprise most of the β^S chromosomes in the Brazilian population.[5] Thus, we were unable to verify whether this region had specific sequence variations in all other β^S haplotypes. To ascertain whether the TAA insertion is present exclusively in β^S-Benin chromosomes it would be necessary to extend the analysis of 3'-HS polymorphisms to a more diversified population sample. Nevertheless, we analyzed β^S-bearing chromosomes in 28 SC and 152 AS individuals. Among 380 β^S chromosomes typed, all β^S-Benin had the novel TAA allele (121 out of 121 chromosomes). Thus, we believe that SSCP analysis is a suitable method for distinguishing sequence variations easily in the 3'-HS AT-rich region. By scoring electrophoretic banding patterns, this specific polymorphism can be a useful tool for rapid resolution of β^S RFLP-haplotypes in the heterozygous state. It is noteworthy that β^S-Bantu, β^C and reference chromosomes have the same 3'-HS nucleotide sequence, whereas the Benin-type β^S chromosome has this unique polymorphism.

Putative recombination hotspots were found to elevate haplotype diversity, which have divided the whole β-globin gene haplotype into two or three subhaplotypes. The available evidence indicates that *recombinogenic* sites are located between the δ-globin and β-globin genes,[7] and between the LCR and structural globin genes.[8-11] Despite the fact that the 3'-HS site is located downstream to an extensive unstable LINE-1 sequence, our results demonstrate that this region is probably not affected by recombination events.

As crossing-over or gene conversion is more frequent than mutations, there is not complete assurance of multiple occurrences of β-globin mutations from RFLP-haplotype studies. In this way, the identification of a polymorphic marker specific for βˢ–Benin haplotype far downstream of recombination sites may clearly be of utility in refining the discriminative power of haplotypes in evolutionary and clinical studies of SCA since the associations of haplotype and phenotype are controversial in this disorder.[11]

Contributions and Acknowledgments

SB designed the study and prepared the manuscript. Together with STOS and FFC, SB analyzed the results and reviewed the manuscript. VGC, ASSD, and AZV performed the RFLP and SSCP analyses. DSB and MBM performed the sequencing of the HS´ repeat region.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor George Stamatoyannopoulos, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Stamatoyannopoulos and the Editors. Manuscript received October 26, 2001; accepted January 3, 2002.

Wat is already known on this topic

Identification of the βˢ gene cluster haplotype might provide a useful tool for the detection of high-risk patients with sickle cell anemia.

What this study adds

This paper reports a TAA insertion in the 3' UTR of the β-globin cluster that appears to be specific for the Benin haplotype.

Potential implications for clinical practice

There are no clinical implications in the short term.

George Stamatoyannopoulos, Associate Editor