Rearrangements of the β-globin gene cluster in apparently typical β^s^ haplotypes

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Background and Objectives. The majority of the chromosomes with the β^g^ gene have one of the five common haplotypes, designated as Benin, Bantu, Senegal, Cameroon, and Arab-Indian haplotypes. However, 5-10% of the chromosomes have less common haplotypes, usually referred to as atypical haplotypes. We have demonstrated that most atypical haplotypes are generated by recombinations. The present study was carried out in order to explore whether recombination also occurs in chromosomes with the common (or typical) haplotypes.

Design and Methods. We screened the HS-2 region of the β-globin gene locus control region (LCR) in 244 sickle cell patients who had typical restriction fragment length polymorphism (RFLP)-defined haplotypes of the β^-g^ gene cluster. For 14 cases in which the expected and the observed LCR repeat-sequence sizes were discrepant, the analysis was extended to other unexplored polymorphic markers of the β^-g^ gene cluster, i.e., pre-γT framework, pre-γT 6-bp deletion, HS-2 LCR (AT)xR(AT)y and pre-β(AT)xTAT repeats, and the intragenic β^-g^ gene framework.

Results. In all 14 cases (15 chromosomes) in which the LCR repeat-sequence sizes were discrepant, a recombination involving a typical 3' segment of the β^-g^ gene cluster was demonstrated. In most of the cases, the recombination site was located between the β^-g^ gene and the β^-CLCR. Nine cases involving recombination were detected among 156 Brazilian HbS homozygotes and five among 88 African patients homozygotes for the Benin haplotype.

Interpretation and Conclusions. Thus, 3.1% of apparently typical haplotypes linked to the sickle cell gene involve recombinations similar to those that generate the atypical haplotypes, a finding that reinforces the picture of the β^-g^ gene cluster as highly dynamic.

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The β^-s^ gene responsible for sickle cell anemia is commonly associated with five different haplotypes, namely Benin, Bantu, Senegal, Cameroon and Arab-Indian. The different haplotypes are usually recognized by a set of polymorphic restriction enzyme profiles of the region that constitutes the β^-g^ gene cluster, and have been named after the geographic regions or ethnic groups in which the designated β^-s^ haplotype was common. ¹⁻³ There is controversy concerning the meaning of this geographic homogeneity of β^-s^ gene haplotypes, demonstrating either the independent origin of the β^-s^ mutation in these regions²⁻⁵ or the single center of the β^-s^ mutation followed by recombination and gene conversion.⁶ In addition to its anthropologic interest, the polymorphisms linked to the β^-g^ gene have also been considered as genetic markers to explain the differences both in the clinical expression of sickle cell anemia and in drug response.³

The majority of the chromosomes with the β^-g^ gene have one of the five common haplotypes, although in every large series of sickle cell patients there is a minority of chromosomes (about 5%) associated with less common haplotypes, usually referred to as atypical haplotypes. We have demonstrated that most of the atypical haplotypes are generated by simple or double crossovers between two typical β^-g^ haplotypes or much more frequently between a typical β^-g^ haplotype and a different β^-g^ associated haplotype.⁷ However, we also observed two cases in which recognition occurred on the background of a common or typical haplotype. Hence, the present study was designed in order to explore how frequent and diverse are recombinations in chromosomes carrying the β^-g^ gene associated with the common (or typical) haplotypes.

Design and Methods

This study comprises 244 unrelated sickle cell anemia patients who had typical β^-g^ haplotypes as determined by classical 7-site restriction polymorphism (RFLP) of the β^-g^ gene cluster; 156 samples were collected from Brazilian sickle-cell patients, and 88 were from...
African patients from Benin. All patients were HbS homozygotes as determined by hemoglobin electrophoresis and by MstII digestion of a β-globin gene segment obtained by polymerase chain reaction (PCR). The β-haplotypes (based on seven restriction sites) were determined by PCR followed by restriction enzyme digestion as previously described.

The (AT)xN12(AT)y repeat sequence configuration within the HS-2 region of the β-locus control region (LCR) was screened by PCR amplification of a 324 bp fragment that was subsequently digested with XbaI and HindII followed by electrophoresis of the product on 7% polyacrylamide for 4 hours at 180V. In addition to the 47, 77 and 87 bp constant fragments, a fragment of variable size between 104 and 120 bp is observed, depending on the size of the (AT)xN12(AT)y repeat. For the Bantu and the Benin typical motifs, 110 and 104 bp fragments, respectively, are obtained.

For the cases in which the expected and the observed LCR repeat-sequence sizes were discrepant, the analysis was extended to other polymorphic markers of the β-gene cluster. PCR-based methods were used to explore further the following 5 regions of the β-globin cluster:

1) the (AT)xTy motif was amplified and directly sequenced according to the procedure described by Trabuchet et al. In heterozygotes, the PCR product was cloned by the TA Cloning kit (Invitrogen, Carlsbad, Ca, USA), and the clones were sequenced with the T7 Sequencing kit (Pharmacia). Within the same segment, the C/T polymorphism at –551 of the β-gene was detected by amplification of a 640 bp segment followed by direct observation of the product size and heterodimer formation on electrophoresis;

2) the 6 bp deletion at –400/395 nt at 5’ to the G γδβ haplotype –403/390 G γδβ haplotype -403/390 G γδβ haplotype –551 (AT)y repeat configuration within the HS-2 region of the β-LCR, a 324 bp fragment was amplified. The PCR product was cloned and sequenced as described above;

3) the pre-γ-gene framework 11 between –1450 and –1225 nt at 5’ to the γ-gene was determined by amplification and direct sequencing;

4) to explore further the (AT)xN12(AT)y repeat sequence configuration within the HS-2 region of the β-LCR, a 324 bp fragment was amplified. The PCR product was cloned and sequenced as described above;

5) the β-globin gene frameworks were defined by DGGE of a 474 bp fragment amplified by primer-pair G as described by Ghanem et al.

Results and Discussion

The expected and the observed LCR repeat-sequence sizes were concordant for 147 patients from Brazil and 83 from Benin. For 14 cases (nine cases from Brazil and five from Benin), the LCR repeat size obtained in the screening procedure was discrepant with the size expected for that specific haplotype (or haplotype combination in the case of heterozygotes). Since the uncommon β-globin cluster structure was homoygous in one case, there were 15 discrepant chromosomes in a total of 488 studied (3.1%). On the basis of 7-restriction site polymorphisms, the haplotypes of these cases had been classified as Benin/Bantu double heterozygotes (5 cases), Bantu homozygotes (2 cases), Benin homozygotes (6 cases) and Benin-Arab-Indian double heterozygote (1 case). In all the 14 chromosomes in which the LCR repeat-sequence sizes were discrepant, a recombination involving a typical 3’ segment of the β-globin cluster was demonstrated (Table 1). The markers within and near the β-gene (Rsal site, (AT)xTy repeat motif and the β-globin gene framework) were always concordant: either Benin-like or Bantu-like. In six samples (cases #3, 4, 6, 7, 8 and 9) the single recombination site was located

Table 1. Extended β-cluster haplotypes of 9 HbS homozygotes from Brazil (cases #1-9) and four from Benin (cases #10-13) with typical RFLP haplotypes who had a discrepant LCR in relation to the RFLP haplotype.

<table>
<thead>
<tr>
<th>Case</th>
<th>β-gene</th>
<th>γδβ cluster</th>
<th>6-site haplotype*</th>
<th>-551 T/C (ψ)</th>
<th>-530 (AT)xTy</th>
<th>Framework</th>
</tr>
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<tr>
<td>1</td>
<td>9N10/9N10</td>
<td>1/1</td>
<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
<td>1/1</td>
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<td>8N7/8N7</td>
<td>1/1</td>
<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
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<tr>
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<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
<td>1/1</td>
</tr>
<tr>
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<td>8N7/8N7</td>
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<td>ND/ND</td>
<td>G/C</td>
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<td>G/C</td>
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<td>G/C</td>
<td>6.9/6.9</td>
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<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
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</tr>
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<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
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<td>ND/ND</td>
<td>G/C</td>
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<tr>
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<td>ND/ND</td>
<td>G/C</td>
<td>6.9/6.9</td>
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<td>G/C</td>
<td>6.9/6.9</td>
<td>1/1</td>
</tr>
</tbody>
</table>

* R: N=ACA CAT ATA GTG and N'=ACA CAT ATA GTG GT; ° 6 bp deletion or non-deletion; # XmnI at 5’ to γ; HindIII at 5’ to ψβ; HindIII at 5’ to ψβ; HindII at 5’ to ψβ; HindII at 5’ to ψβ.
between the HincII restriction site at 5' of the ε-globin gene and the LCR, so that a typical haplotype at the γβ cluster was combined with a repeat sequence within the HS-2 region of the LCR that is not the usual structure observed for that specific haplotype. In cases #3, 4, 6 and 7 the LCR was either Benin-like or Bantu-like, whereas for two individuals (cases #8 and 9) the LCR repeat structure was unusual for the sickle-cell haplotypes. Case #1 was homozygote for a haplotype which was Bantu-like at the 3' region (including the Rsal site, the (AT)xTy repeat motif and the β-globin gene framework), Benin-like at the 5' region of the β-globin cluster (including the pre-εγ framework) and had an LCR similar to that observed in the Senegal haplotype. In case #2 the structure was Bantu-like from the 6 bp deletion region (-400 nt of εγ-gene) downstream. The LCR was Benin-like, but the pre-εγ framework was 4 instead of 1. Case #5 was a double-heterozygote for atypical chromosomes. One chromosome had an LCR HS-2 repeat and a pre-εγ framework of the Cameroon type and a Benin-like haplotype downstream from the εγ-gene. The other chromosome had a Senegal haplotype from the LCR to the HincII site 3' to the γε gene, and a Bantu-like sequence thereon, suggesting a recombination in a region considered as a β-cluster recombination hotspot. This chromosome, when analyzed with only 6-RFLP sites, was erroneously identified as a typical Arab-Indian haplotype. Cases #6 and 9 were identical: one chromosome was typical Bantu, the other was Bantu-like from the ε-globin gene downstream to the βγ-globin gene framework, but the HS-2 repeat sequence at the LCR had the unusual structure of (AT)x7ACACATACGT(AT)x11. Cases #10-14 were from Benin: for each case, one chromosome had a typical Benin haplotype, including the HS2 repeat sequence at the LCR, whereas the other chromosome had a discrepant structure at the LCR, although it was Benin-like from the ε-globin gene downstream to the βγ-globin gene framework. The HS-2 repeat sequence was Senegal-like in two cases, Cameroon-like in one and an uncommon (AT)x7ACACATACGTG(TAT)x6; in the remaining two cases.

We have previously demonstrated that the atypical haplotypes are linked to the sickle cell gene, i.e. the haplotypes that differ from the five most common haplotypes observed worldwide, are produced by diverse genetic mechanisms. These less common haplotypes are generated by recombinations, single nucleotide substitutions or non-reciprocal sequence transfer (conversion) in the pre-existing common haplotypes instead of recurrent de novo βp mutations.

The present work demonstrates that some of the recombinations are not detected by the usual approach of haplotype determination, based on a small number of restriction enzymes sites. As a result, the LCR repeat structure of these cases disagrees with the expected structure in the vicinity of the βγ gene. Since the exact nature of the functional role of the variation of the LCR structure upon β-globin gene expression and severity of the disease are not known, the consequences of these recombinations cannot be established at present. However, there is evidence that this variation may influence the disease severity. Therefore, the determination of the restriction enzyme haplotype is not sufficient to evaluate the phenotype-genotype relationship in sickle cell disease, as for instance when studying the disease severity or the variations in drug response.

In the present study, 3.1% of apparently typical haplotypes involved recombinations. For the same Brazilian sample, 3.9% of atypical haplotypes had been previously observed. For the Benin sample, the percentage of chromosomes with atypical haplotypes was 4/184 (2.2%). Thus, the percentage of uncommon haplotypes in the two populations of sickle cell homozygotes is in the range of 5.3-7.0%. These findings reinforce the picture of the βγ-globin gene cluster as being highly dynamic, and warrant caution about conclusions drawn assuming that the βγ-associated haplotypes are highly stable. Interestingly we found recombinations among both Brazilian patients and African homozygotes for the Benin haplotype, who have a much more homogenous βp-haplotype picture. This result suggests that the recombinations do not solely depend on the diversity of the β and βp haplotypes present in that particular population. This finding should, however, be confirmed in a larger sample of HbS homozygotes and also in other geographic regions where a single major haplotype linked to the βγ-globin gene is prevalent. In conclusion, this study demonstrates that not only atypical but also typical βp haplotypes may involve recombination, a mechanism that can generate diversity of the DNA sequences linked to the βγ-globin gene.

Contributions and Acknowledgments

M A Z designed the study and prepared the manuscript, and together with RK and JE analyzed the results and reviewed the manuscript. SG, IKY and NG performed the RFLP analysis. AGA and MH carried out the analysis of the other polymorphic markers under the supervision of WAS Jr, who also performed the sequencing of the HS-2 repeat region of the LCR.

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Disclosures

Conflict of interest: none.

Redundant publications: none.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous paper (except for 2 cases in a paper published in the AJH, that were the origin of the present investigation).

Manuscript Processing

This manuscript was peer-reviewed by two external referees and by Dr. Carlo Brugnara, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Brugnara and the Editors. Manuscript received November 17, 2000; accepted January 16, 2001.
Potential implications for clinical practice

RFLP haplotyping is not sufficient to define the haplotype of the β-cluster in sickle cell disease. To understand clinical diversity of sickle cell disease it may be necessary to study several molecular markers in the β-globin gene cluster.

References