

# Study of the Single Nucleotide Polymorphism (SNP) at the Palindromic Sequence of Hypersensitive Site (HS)4 of the Human $\beta$ -Globin Locus Control Region (LCR) in Indian Population

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LCR, a genetic regulatory element, was examined in  $\beta$ -thalassemia patients who do not show any mutation in the  $\beta$ -globin genes. We sequenced LCR-HS2, HS3, and HS4 in samples from 16 such patients from the Indian population and found only one SNP A-G in the inverted repeat in HS4. A significant association was observed between the G allele and occurrence of  $\beta$ -thalassemia by Fisher's exact test. The AG and GG genotypes showed higher relative risk as compared to the AA genotype. We also observed linkage disequilibrium between the A/G polymorphism and the AT-rich motif of the LCR HS2 region, suggesting that the G allele could be an evolutionarily new mutation in the study population. *Am. J. Hematol.* 69:77–79, 2002. © 2002 Wiley-Liss, Inc.

**Key words:**  $\beta$ -thalassemia  $\beta$ -globin gene; locus control region; hypersensitive site; single nucleotide polymorphism

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## INTRODUCTION

$\beta$ -Thalassemia is a highly prevalent autosomal recessive disorder characterised by the complete absence of, or some defect in, the  $\beta$ -globin genes, leading to an imbalance of the  $\alpha$ - and  $\beta$ -globin chains [1]. The  $\beta$ -globin LCR, a major regulatory element necessary for high-level transcription of the  $\beta$ -globin genes located approximately 5–20 kb upstream of the  $\epsilon$  gene [2]. LCR consists of five DNase I –HS1–5. Genetic variation in the binding sites of the core fragments of the  $\beta$ -LCR HS 2, 3, and 4 may play a role in cases of  $\beta$ -thalassemia (both major and carrier phenotypes) in which none of the known mutations is found in the  $\beta$ -globin gene. In this study, we sequenced the 1.8-kb region of the  $\beta$ -globin and  $\beta$ -LCR-HS 2, 3, and 4 of the uncharacterized patients in our sample in an effort to discover new polymorphisms that may be associated with the disease and aid in carrier detection.

## SUBJECTS AND METHODS

### Subjects

1,800  $\beta$ -thalassemia patients with varying disease severity were recruited from three centres in northern, eastern, and western India after diagnosis. Control blood samples were collected from different communities with informed consent.

### Phenotype Analysis

Genomic DNA was isolated from whole blood in anticoagulant (EDTA) by using proteinase K–phenol–chloroform standard procedure [3]. Hemoglobin

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variants were analysed by agarose gel electrophoresis at pH 8.6; HbA<sub>2</sub> was determined by gel elution and HbF by alkali denaturation, respectively [4].

### Mutation Analysis and Examination of $\beta$ -LCR HSs

ARMS-PCR method was used to detect known 23  $\beta$ -thalassaemia mutations [3,5].  $\beta$ -globin gene cluster locus control 5' HS 2, 3, and 4 (~460 bp, ~446 bp, and ~442 bp from GeneBank coordinates 8757–9217, 4397–4991, and 668–1109) were sequenced by the ABI Prism 377 automated DNA sequencer using dye terminators chemistry. Sequences were aligned with the corresponding wild-type sequences using Factura and Sequence Navigator program. Statistical analysis was done using Fisher's exact test, genotype relative risk (GRR), and  $\chi^2$  test.

## RESULTS AND DISCUSSION

Of the 1,800  $\beta$ -thalassaemia patients screened by ARMS-PCR 27 did not show any of the 23 known common mutations in the  $\beta$ -globin gene in the Indian population. By direct sequencing of the 1.8-kb region of  $\beta$ -globin gene of these 27 samples, 11 were found to have rare mutations (2 had codon 29(C→T), 2 had codon 39(C→T), and 6 had codon 110 (T→C)), while 16 showed no mutation. Analysis of the core fragment of LCR HS2, 3, and 4 was carried out in these 16 samples in search of novel mutations associated with the disease phenotype.

DNA sequencing of HS2, 3, and 4 core sequences showed only one polymorphism, an A-G, in the palindromic sequence, TGGGGACCCCA, of LCR HS4, in some of the uncharacterised samples. Using samples from 86 normal control subjects and 47 patients ( $\beta$ -thalassaemia major and carriers), we tested for association of this SNP with  $\beta$ -thalassaemia. The allelic frequency of A was 0.76 in controls and 0.63 in patients. We found significant association between the G allele and occurrence of the disorder (Fisher's exact test,  $P = 0.015$ ). The genotypes AA, AG, and GG occurred in 34.0%, 57.5% and 8.5%, respectively, of patients and 55.8%, 40.7%, and 3.5%, respectively, of control individuals. Although this SNP has been reported earlier by Siroongrueng et al. [6], this is the first time that the GG homozygote has been detected in a population. The genotype relative risk (1.49 with 95% confidence interval = (1.09, 2.04)) for genotypes GG and AG suffering from  $\beta$ -thalassaemia as compared to the AA genotype is higher, which confirms

**TABLE I. Linkage Disequilibrium Between SNP at the Palindromic Sequence in LCR-HS4 and (AT)<sub>x</sub>N<sub>y</sub>(AT)<sub>z</sub> Length Polymorphism in LCR-HS2<sup>a</sup>**

(AT) <sub>x</sub> N <sub>y</sub> (AT) <sub>z</sub> polymorphism	SNP	
	A	G
(AT) <sub>10</sub> N <sub>12</sub> (AT) <sub>11</sub>	10 (33.33)	0 (0)
(AT) <sub>9</sub> N <sub>12</sub> (AT) <sub>11</sub>	2 (6.67)	0 (0)
(AT) <sub>9</sub> N <sub>12</sub> (AT) <sub>10</sub>	2 (6.67)	16 (53.33)

<sup>a</sup>Number of haplotypes at the two loci (percentage frequency of the haplotype) are shown in the table.

the expectation from the allelic association of G with disease.

We observed G allele in the HS4 locus is associated with only one distinct pattern (AT)<sub>9</sub>N<sub>12</sub>(AT)<sub>10</sub> of HS2 of the  $\beta$ -LCR (Table I), showing linkage disequilibrium between the two loci and suggesting that the G allele could be an evolutionarily new mutation in the Indian population. The occurrence of A in the middle of a short palindromic sequence causes the formation of a hairpin structure [7]. Replacement of A by G decreases the stability of this structure [7], and it may be responsible for the observed low frequency of the G allele. However, it does not by itself explain how this may play a role in  $\beta$ -thalassaemia. Although structural variations caused by the replacement of A by G might not have a direct casual effect, it is possible that it modifies gene expression [8,9] and thus the disease severity.

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