Letter to the Editor

Presence of atypical beta globin (HBB) gene cluster haplotypes in sickle cell anemia patients of India

Dear Editor,

Sickle cell disease (SCD) is an autosomal recessive disease caused by a transversion type of point mutation in the beta globin (HBB) gene. Sickle cell anemia in India is common among the ethnic population groups with the highest prevalence being seen among the caste and tribal populations of Central and South India.¹ The HBB gene cluster includes the embryonic ε gene, the two fetal globin genes Gγ and Aγ, a δ pseudo-gene, the δ gene and finally the HBB gene itself. The HBB gene cluster is located on the short arm (p) of chromosome 11 and spans a region of 70 kilobases. Identification of polymorphic restriction endonucleases sites in and around the HBB gene has led to the delineation of the five HBB gene cluster haplotypes of sickle cell anemia.² These haplotypes of sickle cell anemia are important as population data, for anthropological purposes to trace the migration of the sickle cell gene and to monitor the clinical severity of the disease.³ Given the fact that there are significant differences in the clinical and hematological variables among populations, it is important to characterize haplotypes to understand the clinical and phenotypic heterogeneity of this devastating disease. Hence, the present study is aimed at determining the prevalence of HBB gene cluster haplotypes in the Chhattisgarh population.

One hundred unrelated random SCD-hemoglobin (Hb) SS patients (56 males and 44 females) receiving treatment in the outpatient department of the Sickle Cell Institute, Chhattisgarh, Raipur, were enrolled. The Institutional Ethics Committee of Pandit Jawahar Lal Nehru Memorial Medical College, Raipur, approved the study. Informed written consent was obtained from study participants. A 3 mL blood sample was collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted using a standard protocol.⁴ DNA sequences encompassing the relevant HBB gene cluster polymorphic variant sites were amplified by polymerase chain reaction (PCR) and the fragments were subjected to restriction fragment length polymorphism (RFLP). Eight RFLP sites (5′ to ε,⁵ 5′ to Gγ,⁶ IVS II Gγ,⁶ IVS II Aγ,⁶ 5′γβ,⁷ 3′β,⁷ 5′β,⁸ and IVS II β) were analyzed to identify five known global HBB haplotypes.

As phase-unknown genotypes were collected, the haplotype sites and frequencies were estimated using maximum likelihood with an expectation-maximization method in Arlequin 3.5 software. The descriptive data are reported as means ± standard deviation (SD). The statistical analysis of the study data was performed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows, Version 22.0.

Analysis of HBB haplotypes using PCR-RFLP revealed four haplotypes, Arab-Indian (+, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, +, 180-182
and in areas of Mid-Western Africa. Haplotypes specific to a certain geographical region are suggestive of the mutant HBB gene arising separately in these locations. However, several studies reported the presence of Senegal, Benin, Bantu, and Cameroon haplotypes in Indian populations. The atypical HBB haplotypes observed might have originated from the pre-existing common haplotypes, due to shuffling of pre-existing polymorphisms during meiotic recombination. Rearrangements of the HBB gene cluster in apparently typical HBB haplotypes has been demonstrated in several studies. Atypical haplotypes have also been reported in several studies. Fifteen atypical haplotypes from 20 different haplotypes have been identified in Relli and Thurpu Kapu populations of Andhra Pradesh. In summary, the analysis of haplotypes revealed the presence of four haplotypes. Although our study includes more SCD patients compared to previous studies, keeping the diversity of Indian populations in mind, our results should be considered preliminary and replication of haplotype analysis should be performed in much larger samples from other regions of the Indian sub-continent.

Conflicts of interest
The authors declare no conflicts of interest.

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References

Table 1 - Hematological profile of study participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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</thead>
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<tr>
<td>Hb (g/dL)</td>
<td>9.10 ± 2.20</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>20.70 ± 6.78</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>26.08 ± 6.98</td>
</tr>
<tr>
<td>TLC (×10^12/L)</td>
<td>10.87 ± 10.86</td>
</tr>
<tr>
<td>Platelet count (×10^12/L)</td>
<td>318.99 ± 166.59</td>
</tr>
<tr>
<td>RBC count (×10^12/L)</td>
<td>3.10 ± 0.72</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>83.19 ± 12.09</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>34.20 ± 1.92</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28.58 ± 3.90</td>
</tr>
<tr>
<td>DLC (%)</td>
<td>42.35 ± 14.31</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation.

Hb: hemoglobin; Hb F: fetal hemoglobin; TLC: total leukocyte count; RBC: red blood cell; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; MCH: mean corpuscular hemoglobin; DLC: differential leukocyte count.

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