



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 $G\gamma$ and $A\gamma$, a ψ 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 $G\gamma$,⁶ IVS II $G\gamma$,⁶ IVS II $A\gamma$,⁶ 5' ψ β ,⁷ 3' ψ β ,⁷ 5' β ,⁸ and IVS II β ,⁶) 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 \pm 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 (+, +, +, -, +, +, -, +, +), Senegal (-, +, +, -, +, +, +, +, +), Benin (-, -, -, -, -, +, -, +, +) and Bantu/Central African Republic (-, -, +, -, -, -, -, +, +). We did not find the Cameroon haplotype in our study subjects. The Arab-Indian haplotype was the most common haplotype (78%) followed by atypical haplotypes (15%). Senegal, Benin and Bantu haplotypes were found in 4%, 2% and 1% of these sickle cell disease patients, respectively. High prevalences of two atypical haplotypes (+, -, +, -, +, -, +, + and +, +, +, -, +, +, +, +) indicated that these haplotypes are common among the samples we examined. The atypical haplotype structure detected in the sample might have been generated through a recombination process. Hematological parameters in our study subjects are documented in Table 1. The mean hemoglobin concentration in the study group is slightly lower than the normal range of 11.0–16.0 g/dL. The mean Hb F concentration of the Arab-Indian haplotype was $20.70 \pm 6.78\%$, which is significantly higher than reported in previous studies from India and abroad.⁹⁻¹¹

The Arab-Indian haplotype is one of the major *HBB* haplotypes that show different clinical and hematological profiles compared to the other haplotypes.¹² The other less frequent haplotypes in India, such as Senegal, Bantu, Benin and Cameroon are mainly seen in African SCD patients. The Senegal haplotype is most prominently seen in Senegal and in the Atlantic-Western region of Africa.¹³ The Bantu haplotype is found in the entire Northern Africa region and in the area of the Mediterranean Sea, and is probably of Central-West African ancestry.¹⁴ The Cameroon haplotype is seen commonly among ethnic groups of Cameroon and also in areas along the west coast of Africa.¹⁵ The Benin haplotype is found most commonly in Nigeria and Benin

Table 1 – Hematological profile of study participants.

| Variable | Value |
|---|-----------------|
| Hb (g/dL) | 9.10 ± 2.20 |
| Hb F (%) | 20.70 ± 6.78 |
| Hematocrit (%) | 26.08 ± 6.98 |
| TLC ($\times 10^9 L^{-1}$) | 10.87 ± 10.86 |
| Platelet count ($\times 10^9 L^{-1}$) | 318.99 ± 166.59 |
| RBC count ($\times 10^{12} L^{-1}$) | 3.10 ± 0.72 |
| MCV (fL) | 83.19 ± 12.09 |
| MCHC (g/dL) | 34.20 ± 1.92 |
| MCH (pg) | 28.58 ± 3.90 |
| DLC (%) | 42.35 ± 14.31 |

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.

and in areas of Mid-Western Africa.¹⁶ Haplotypes specific to a certain geographical region are suggestive of the mutant *HBB* gene arising separately in these locations.¹⁷ However, several studies reported the presence of Senegal,¹⁸ Benin,¹⁰ Bantu,¹² and Cameroon¹⁰ 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.¹⁹ Atypical haplotypes have also been reported in several studies.^{11,12,20} Fifteen atypical haplotypes from 20 different haplotypes have been identified in Relli and Thurpu Kapu populations of Andhra Pradesh.¹¹

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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