Fetal Hemoglobin Levels and $\beta^S$ Globin Haplotypes in an Indian Population With Sickle Cell Disease

By A.E. Kulozik, B.C. Kar, R.K. Satapathy, B.E. Serjeant, G.R. Serjeant, and D.J. Weatherall

To further explore the cause for variation in hemoglobin F (Hb F) levels in sickle cell disease, the $\beta^S$ globin restriction-fragment length polymorphism haplotypes were determined in a total of 303 (126 SS, 141 AS, 17 S$^+$, 7 A$^+$, and 12 AA) Indians from the state of Orissa. The $\beta^S$ globin gene was found to be linked almost exclusively to a $\beta^S$ haplotype (+++++---), which is also common in Saudi Arabian patients from the Eastern Province (referred to as the Asian $\beta^S$ haplotype). By contrast, the majority of $\beta^S$ and $\beta^A$ thalassemia globin genes are linked to haplotypes common in all European and Asian populations (+---+---; --+++++). Family studies showed that there is a genetic factor elevating Hb F levels dominantly in homozygotes (SS). This factor appears to be related to the Asian $\beta^S$ globin haplotype, and a mechanism for its action is discussed. There is also a high prevalence of an independent Swiss type hereditary persistence of fetal hemoglobin (HPFH) determinant active in both the sickle cell trait and in sickle cell disease.

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The clinical course of homozygous sickle cell (SS) disease varies from death in infancy to absence of symptoms with almost normal life expectancy.1 An important determinant of clinical severity is the level of fetal hemoglobin (Hb F) that inhibits intracellular polymerization of sickle hemoglobin (Hb S), and hence sickling, and reduces the severity of hemolysis and the occlusion of the microcirculation. A recent study has shown that SS disease in Orissa State, India has a mild clinical picture associated with high levels of Hb F and a high prevalence of $\alpha$ thalassemia.2 Classical and molecular genetic analyses in this population have produced evidence for a dominant factor linked to the $\beta^S$ globin gene that is responsible for increased $\gamma$-globin gene expression in both SS disease and sickle cell $\beta^S$ (S$^B\beta^S$) thalassemia. A separate Swiss type HPFH,3 active in both heterozygotes (AS) and homozygotes (SS), appears to be common in these families.

PATIENTS AND METHODS

During a three-week period in February/March 1986, clinical and hematologic examinations were performed on 148 patients with sickle cell disease (131 SS; 17 S$^+$ thalassemia). In addition, 142 persons with the sickle cell trait, seven with the $\beta^A$ thalassemia trait and 12 persons with a normal globin genotype who were parents or siblings of the index cases, were also studied. Relatives below the age of 3 years were excluded because of the effect of young age on Hb F. Peripheral blood samples were kept frozen at $-20^\circ$C and within 3 weeks were transported to Oxford where $\gamma$- to $\gamma$ globin chain ratios were measured with high pressure liquid chromatography4 and where DNA was extracted. Further aliquots of the peripheral blood samples were kept at 5°C to 10°C and were taken to Jamaica where Hb F levels were measured by alkali denaturation,5 for which the upper limit of normal for that laboratory was 0.7%. DNA was digested with restriction endonucleases Hind II, Hind III, Xmn I, and Bam HI under conditions recommended by the manufacturers. The $\beta$ globin gene haplotype was determined by Southern blot analysis6 using the following genomic DNA fragment probes: 1.3 kb Bam HI/EcoRI c; 3.3 kb Hind III y; 1.8 kb Bgl II/Xba I $\psi$$\beta$; Bam HI/EcoRI IVS-2 $\beta$. In cases with two different haplotypes the correct haplotype pair was derived by family studies, but in one patient with SS disease and in eight with S$^A\beta$ thalassemia this was not possible, and it was assumed that a common haplotype was present with a rare haplotype rather than two rare haplotypes.

RESULTS

Some clinical and hematologic characteristics of the SS patients are reported elsewhere.2

Hemoglobin F levels. The levels of fetal hemoglobin in Indian SS patients are markedly higher than in Jamaican patients.2 Mean Hb F levels did not differ significantly between the age groups of 2 to 5 years (17.1%), 6 to 10 years (17.7%), and 11 to 20 years (17.5%) but were significantly lower in those above 20 years (14.5%). Hb F levels in Indian patients with S$^A\beta$ thalassemia were markedly higher (mean 14.9%) than in a comparable Jamaican group7 but did not differ significantly ($P > 0.1$) from Indian SS patients. $\alpha^S/\gamma$ ratios in ten SS patients with Hb F levels of about 20% ranged from 0.68 to 0.82; three SS patients with Hb F levels below 10% showed ratios of 0.82, 0.84, and 0.85; and in seven other such patients ratios were above 0.6, although in these a more precise quantitation was not possible.

The mean Hb F level in 108 AS adults (20 years and above) was 0.7% and in 33 nonadults (3 to 19 years) was 0.9%. Hb F levels exceeded 1% in 25 (23%) AS adults and in nine (27%) AS nonadults. Comparison of the Hb F distribution in Indian and Jamaican AS parents with SS offspring (Fig 1) indicated that high levels occur more frequently in Indian parents. The mean Hb F level in 12 AA relatives was 0.3% (range 0.1% to 0.6%).

Family studies. Hb F levels in Jamaican patients with SS disease are known to be related to the Hb F levels in their

From the MRC Molecular Haematology Unit, Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, England; the Department of Medicine and Department of Paediatrics, VSS Medical College, Burla, Orissa, India; and MRC Laboratories (Jamaica), University of the West Indies, Mona, Kingston, Jamaica.

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Address reprint requests to Dr. A.E. Kulozik, MRC Molecular Haematology Unit, University of Oxford, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, England.

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heterozygous AS offspring have a phenotype similar to the Swiss type HPFH with Hb F levels of more than 1%. This phenotype seems to segregate in a Mendelian fashion but independently of the $\beta$ haplotype.

**Haplotype analysis.** With the combination of enzymes and probes used in the present study, the commonest $\beta^A$ haplotype was $+++-++-+$ (Hind II $\epsilon$, Xmn I $\gamma$, Hind III $\alpha'$, Hind II $\delta$, Hind II $\psi$, Hind II $3'$ $\psi\beta$, Bam HI $\beta$) and closely resembles that designated No. 31 by Antonarakis et al. Since different restriction sites were used and since the haplotype is commonly linked to the $\beta^A$ globin gene in Saudi Arabian$^{10,14}$ and Indian$^{10,14}$ but not in African populations, it is referred to as the Asian $\beta^A$ haplotype.

In 126 SS patients analysis of the $\beta^A$ haplotypes revealed that 107 (85%) were homozygous for the Asian $\beta^A$ haplotype, 17 (13%) were heterozygous, and two patients had a haplotype that differed only at the Hind II $\epsilon$ site. The distribution of Hb F levels did not differ significantly between these groups (Table 1). Among 141 AS individuals the $+++-++-+$ haplotype occurred homozygously in seven, heterozygously in 120, and did not occur on either chromosome in 14 persons (Fig 3). Mean Hb F levels in these groups expressed as the geometric means were 1.49%, 0.63%, and 0.41% respectively; analysis of variance indicated a highly significant trend ($P = 0.0002$) between groups. Hb F levels exceeded 1% in five (71%) homozygotes, in 27 (23%) heterozygotes, and in two (14%) individuals without this haplotype.

The frequency of $\beta^A$, $\beta^A$, and $\beta^A$ thalassemia haplotypes in unrelated individuals (Table 2) indicates that the $\beta^A$ mutation occurs predominantly on the Asian haplotype and $\beta^A$ thalassemia mutations on the $+++-++-+$ haplotype. Normal $\beta$ globin genes were carried on chromosomes characterized by a variety of different haplotypes, most frequently the $+++-++-+$ or the $+++-+++$ pattern.

**DISCUSSION**

Some of the clinical variability of SS disease has been attributed to raised Hb F levels causing a milder clinical

| Table 1. $\beta$ Globin Haplotypes, Hb F Levels, and Ages of Indian Patients with SS Disease and $S\beta^A$ Thalassemia |
|---|---|---|---|---|
| Haplotype | Genotype | Mean Hb F | Mean Age |
| SS | $+++-++-+$ | 107 | 16.4 | 16.6 |
| | $+++-++-+$ | 15 | 15.5 | 13.3 |
| | $+++-++-+$ | 1 | 19.3 | 4 |
| | $+++-++-+$ | 1 | 22.3 | 4 |
| | $+++-++-+$ | 1 | 24.5 | 18 |
| | $+++-++-+$ | 1 | 10.5 | 8 |
| $S\beta^A$ | $+++-++-+$ | 15 | 14.3 | 20.2 |
| | $+++-++-+$ | 1 | 18.4 | 24 |
| | $+++-++-+$ | 1 | 18.7 | 13 |

The map represents the $\beta$ globin gene cluster with restriction endonuclease dimorphisms used in this study.

Abbreviations: H, Hind II; Hd, Hind III; B, Bam HI; X, Xmn I; +, presence of the respective restriction site; -, absence of the respective restriction site.
Elevated Hb F levels with a mild clinical phenotype appear common in Arab and Iranian patients. The limited data previously available suggested that elevated Hb F levels were also common in Indian patients. Selected family studies have suggested that the high Hb F levels might be due to an HPFH determinant linked to the $\beta^\alpha$ globin gene and associated with a particular $\beta^\alpha$ haplotype $(+++---)$. Furthermore, the geographic distribution of this haplotype coincides with the occurrence of high Hb F levels and mild SS disease.

In the present study of a large number of Indian patients with SS disease and $S^{\delta\beta}$ thalassemia, the Hb F level was found to be raised and the $\beta^\alpha$ globin gene was almost exclusively linked to the Asian $\beta^\alpha$ haplotype. The basis of this relationship is unclear. Among AS individuals there was evidence that Hb F levels were directly related to the number of copies of the Asian $\beta^\alpha$ haplotype inherited, consistent with a genetic determinant linked to this haplotype. However, the SS population was so homogeneous (85% being homozygous

![Table 2. Haplotype Frequencies of Independent $\beta^\alpha$, $\beta^\beta$, and $\beta^\delta$](image)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>$\beta^\alpha$</th>
<th>$\beta^\beta$</th>
<th>$\beta^\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$+++-++$</td>
<td>187 (89.9%)</td>
<td>5 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>$-++-++$</td>
<td>18 (8.6%)</td>
<td>1 (0.5%)</td>
<td></td>
</tr>
<tr>
<td>$--++-+-$</td>
<td></td>
<td>1 (0.5%)</td>
<td>23 (19.3%)</td>
</tr>
<tr>
<td>+--------+</td>
<td>1 (0.5%)</td>
<td>10 (83.4%)</td>
<td>56 (47.1%)</td>
</tr>
<tr>
<td>$-+$-----</td>
<td>2 (1.0%)</td>
<td>8 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>7 (5.9%)</td>
<td></td>
</tr>
</tbody>
</table>

Totals 208 12 119

The map is as in Table 1. Other haplotypes ($++---++-$; $+----+++--; +++++++$) were observed on one chromosome only.

for the Asian $\beta^\alpha$ haplotype) that it was unsuitable for examining whether this haplotype was linked to factors determining Hb F level. Indeed, only two patients did not possess this haplotype, but in both there was a closely related haplotype. It is conceivable that in this population the Hb F levels observed in SS disease are not linked to the Asian $\beta^\alpha$ haplotype but reflect some other genetic factor common in Asian Indians that facilitates increased $\gamma$-globin gene expression under erythropoietic stress. However, most patients with homozygous $\beta$ thalassemia of mixed Asian Indian origin are transfusion dependent with low Hb F levels, and the genetic background is similar to Orissan Indians as the common $\beta^\alpha$ haplotypes in these two populations are identical (Table 2). It is of interest that patients with homozygous $\beta$ thalassemia with a haplotype closely related to the Asian $\beta^\alpha$ haplotype have higher Hb F levels and that this observation together with the data on the Asian $\beta^\alpha$ haplotype in this report support the hypothesis that a determinant for raised Hb F levels is linked to that haplotype. Furthermore, the HPFH factor linked to the Asian $\beta^\delta$ haplotype seems to be dominant in SS disease and $S^{\delta\beta}$ thalassemia (Table 1), as one copy of the Asian $\beta^\delta$ haplotype is sufficient to raise the Hb F level. The mechanism for this effect is unclear. One possibility is a gene closely linked to the Asian haplotype that encodes for a factor acting in trans on both chromosomes. Alternatively, a site, such as a promoter region, within the Asian haplotype might be capable of locally concentrating transacting factors that might then influence the paired homologous allele. This latter mechanism has been suggested in drosophila where transcriptional enhancement of the Sgs-4 gene was shown to be mediated by diffusible factors that are at a high local concentration and can be utilized by paired homologous genes within the same domain.

It has been shown previously that a C→T mutation at position −158 5′ to the $\gamma^\delta$ globin gene that introduces an Xmn I restriction site is linked to the core haplotype $++-++$ (Hind III$\gamma$, Hind II$\gamma\beta$) and that it is associated with a raised $\gamma^\delta/\gamma^\alpha$ globin chain ratio. This site is present in the common Indian $\beta^\delta$ haplotype, confirming the tight linkage of the $\gamma^\delta/\gamma^\alpha$ chain ratios. The lack of SS patients not carrying the $\gamma^\delta - 158$ C→T mutation in this study—only three are heterozygous and all the others homozygous for this mutation—unfortunately does not allow us to address the question whether the $\gamma^\delta - 158$ C→T mutation causes raised Hb F levels in Indian patients with SS disease.

Whatever mechanism is involved, inheritance of the Asian $\beta^\delta$ haplotype is not the only determinant of Hb F levels in this population. In some families there appeared to be an HPFH determinant, phenotypically of the Swiss type, which segregated independently of the Asian $\beta^\delta$ haplotype. Thus raised Hb F levels in AS offspring segregated in families where at least one parent had a high Hb F level and even within the SS population who were mostly homozygous for the Asian $\beta^\delta$ haplotype, the mean Hb F differed by a statistically significant 3%, depending on whether an AS parent manifested a raised Hb F level. There appear, therefore, to be at least two
determinants of the Hb F level in this Indian population. An HPFH determinant that has also been recognized in Jamaicans accounts for the increasing Hb F levels observed with increasing parental Hb F levels. However, for each level of parental Hb F, the Hb F observed in Indian SS offspring exceeds that in comparable Jamaicans, and this difference may be related to possession of the Asian β⁺ haplotype.

Of the three β⁺ globin haplotypes recognized in homozygous sickle cell disease in Africa, only one, designated the Senegal haplotype, was associated with high Hb F levels. The close similarity between the Senegal haplotype (- + + + + + +) and the Asian β⁺ haplotype (+ + + + + + + + + + +) is therefore of interest. The characterization of factors responsible for raised Hb F levels is clearly important in understanding the natural history of sickle cell disease, is of clinical prognostic interest, and is likely to shed some light on mechanisms of globin gene regulation. The Asian β⁺ haplotype (+ + + + + + + + + + + + + + + + + +) seems to be linked to such an HPFH determinant; confirmation of this would require an Asian population with greater heterogeneity of β⁺ haplotypes, allowing study of the relationship of Hb F to different combinations.

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**REFERENCES**

24. Milner PF, Leibfarth JD, Ford J, Barton BP, Grenett HE, Garver FA: Increased Hb F in sickle cell anemia is determined by a factor linked to the β⁺ gene from one parent. Blood 63:64, 1984
29. Gilman JG, Huisman THJ: Two independent genetic factors
in the β-globin gene cluster are associated with high γγ-levels in the Hb F of SS patients. Blood 64:452, 1984


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AE Kulozik, BC Kar, RK Satapathy, BE Serjeant, GR Serjeant and DJ Weatherall