High Fetal Hemoglobin Production in Sickle Cell Anemia in the Eastern Province of Saudi Arabia Is Genetically Determined

By Barbara A. Miller, Mohammed Salameh, Mohammed Ahmed, Jim Wainscoat, Giovanna Antognetti, Stuart Orkin, David Weatherall, and David G. Nathan

Homozygous sickle cell disease in the eastern province of Saudi Arabia is clinically mild. Circulating fetal hemoglobin levels of 16.0 ± 7.4% were found in these anemic patients, but only 1.09 ± 0.97% in their sickle trait parents. To determine whether these sickle cell anemia patients inherit an increased capacity to synthesize fetal hemoglobin, a radioimmunoassay of fetal and adult hemoglobin was performed on erythroid progenitor (BFU-E)-derived erythroblasts from Saudi Arabian sickle cell patients and their parents. Mean fetal hemoglobin content per BFU-E-derived erythroblast from Saudi Arabian sickle cell patients was 6.2 ± 2.4 pg/cell or 30.4 ± 8.6% fetal hemoglobin (normal 1.1 ± 0.7 pg/cell and 5.1 ± 1.8%). Linear regression analysis of % HbF in peripheral blood versus % HbF per BFU-E-derived cell showed a positive correlation with an r of 0.65. The variance of the intrinsic capacity to produce HbF may account for almost 40% (r²) of the variability of circulating fetal hemoglobin but other factors, particularly selective survival of F cells, must also contribute significantly. Despite virtually normal HbF levels in sickle trait parents of these Saudi patients, mean fetal hemoglobin production per BFU-E-derived erythroblast in these individuals was elevated to 3.42 ± 1.79 pg/cell or 16.1 ± 6.4% fetal hemoglobin, and the magnitude of fetal hemoglobin production found in parents correlated with that of the patients. These data indicate that the high fetal hemoglobin in Saudi sickle cell disease is genetically determined but expressed only during accelerated erythropoiesis. Further evidence of such genetic determination was provided by analysis of DNA polymorphisms within the β-globin gene cluster on chromosome 11. This revealed a distinctive 5′-globin haplotype (+ + − + + +) on at least one chromosome 11 in all high F SS and AS tested. The precise relationship of this haplotype to HbF production in this population remains to be defined.

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Materials and Methods

Blood samples. Blood samples were obtained from Saudi Ar- abians who are employees of the Arabian American Oil Company (Aramco) or from their dependents. While patients were chosen at random, no one was included who had received a blood transfusion in the past six months, who had a sickle cell crisis, or active infection in the seven days before assay. All patients were older than three years of age. In each family, at least one member had sickle cell anemia. Blood and bone marrow samples were also obtained from American sickle cell patients seen in the Hematology Clinic at Children’s Hospital, Boston, Mass, and from normal volunteer donors who gave their informed consent. The samples from Saudi Arabia were drawn in 10% ACD and shipped at 4 °C. All assays were performed within 72 hours.

Analysis of blood. CBC and reticulocyte counts, F cell enumeration by acid elution, hemoglobin electrophoresis on citrate agar and cellulose acetate, and alkali denaturation to determine percent fetal hemoglobin were performed on all samples. Ratio of Gγ to Αγ fetal hemoglobin was determined by Triton X-100 electrophoresis. Methylcellulose culture of peripheral blood and bone marrow mononuclear cells. Blood and marrow samples were diluted with tissue culture medium (Iscove’s Modified Dulbecco’s medium—IMDM), layered over Ficoll-hypaque (Pharmacia, Piscataway, NJ), and centrifuged for 20 minutes at 1,200 g at room temperature. The cells at the interface were washed three times. Cells were plated at 1
to 2 x 10^5 cells/mL in 35mm petri dishes (Falcon, Oxnard, Calif) in a mixture containing 0.9% methyldextrinlose, 30% fetal calf serum (Gibco, Grand Island, NY), 9.0 mg/mL deionized BSA (fraction V, Sigma, St. Louis), 1.4 x 10^{-4} mol/L beta-mercaptoethanol, 5% Mo cell line-conditioned medium as a source of BPA (generously provided by Dr David Golde), and with 2.0 U/mL crude erythropoietin (Toyobo, New York) in IMDM. The plates were incubated in humidified 4% CO2 at 37 °C for 14 days and colonies were then counted.

Sample preparation for hemoglobin assay. Erythroid colonies were “plucked” with a drawn out Pasteur pipet, pooled in 0.3 to 0.5 mL IMDM, 4 °C, and the number of cells counted. A precisely measured volume of 0.5 mg/mL carrier sheep HB containing 0.02% Na-azide. This suspension was sonicated, microcentrifuged to remove cell debris, and stored at 4 °C. Samples were routinely assayed within 10 days of preparation.

Radioligand immunoassay of HbA and HbF. The radioligand immunoassay of total hemoglobin or hemoglobin F in pg per BFU-E-derived cell or of percent HbF in such cells was performed as previously described. Briefly, adult and fetal hemoglobin standards were prepared from adult blood, cord blood, or sheep blood samples by CCl4 lysis of washed cells. The adult and cord blood samples were used to construct standard curves and were diluted with 0.5 mg/mL sheep HB. Sonicated cell extracts derived from erythroid colonies diluted with 0.5 mg/mL sheep HB were assayed at two to three dilutions. Antihemoglobin F and total antibodies were prepared from guinea pigs injected with cord blood or alkali denatured cord blood.

Linear standard curves were consistently obtained, and the Hb in 3,000 to 10,000 cells was routinely assayed. Reproducibility of this assay has been discussed previously, but in general, duplicate dishes or multiple assays performed on samples from one individual on different days yielded results within 10% of the mean. The calculation of hemoglobin content per BFU-E-derived cell was based as before on the assumption that all such cells are potential F cells.

Significance of differences among the means of n groups (Tables 2 and 3, n = 5 and 3, respectively) was determined by one-way analysis of variance. A priori comparisons of means of any two groups were then performed, using F tests as tests of significance. A probability of less than 0.05 was taken to be statistically significant. Significance of correlation coefficients (Fig 1, n = 17; Fig 3, n = 15) was assessed using a t distribution.

Restriction endonuclease analysis of DNA and determination of the regional haplotype. Procedures for DNA isolation, blot hybridization, and probes were as previously described. Only five restriction endonuclease site polymorphisms were studied, all in the 5’ region of the β-globin gene cluster. These included a potential Hinc II site 5’ to the e gene, a Hind III site in the intervening sequence 2 (IVS-2) of the Gγ-globin gene, a Hind III site in the IVS-2 of the Aγ-globin gene, and two Hinc II sites, one in the γβ1 gene and another 3’ to it. The “haplotype” for this region was then established, based on the presence or absence of digestion at these sites.

RESULTS

Characterization of patients. Peripheral blood samples were obtained from Saudi Arabian sickle cell anemia patients and their families who met the criteria discussed in Materials and Methods. The results of blood and reticulocyte counts, hemoglobin electrophoresis, F cell determination, and analysis of percent fetal hemoglobin by alkali denaturation are shown in Table 1 and confirm results published elsewhere.1,3,17,18 Mean MCVs were low in Saudi Arabian patients, probably secondary to the high incidence of alpha thalassemia trait17,19,20 and iron deficiency in this region. Fetal hemoglobin levels in the Saudi patients had a mean of 16.0 ± 7.4% compared to 7.1 ± 4.0% for American sickle cell patients of African origin seen in the clinic at Children’s Hospital, Boston. Fetal hemoglobin levels in the Saudi AS patients were generally normal but were elevated in a few parents, as demonstrated by the high standard deviation.

Analysis of fetal hemoglobin from eight Saudi Arabian SCA patients by Triton X-100 gel revealed that all patients had more Gγ than Aγ fetal hemoglobin. The mean value was 76.5 ± 5.1% G gamma globin, a finding previously noted in this population.1 In contrast, 80% of American black patients with sickle cell disease have 40% Gγ and 60% Aγ; only 20% have more Gγ than Aγ.21

Hemoglobin F program in BFU-E–derived erythroblasts. Bone marrow samples were not obtained from Saudi patients. Therefore it was necessary to ascertain that the HbF content of peripheral blood BFU-E–derived erythroblasts was representative of the HbF program. To do so, the total hemoglobin and HbF contents of BFU-E–derived erythroblasts were measured in BFU-E–derived colonies cultured from the bone marrow and peripheral blood of normal consenting American donors and American black sickle cell patients. The results shown in Table 2 confirmed the previous findings of Friedman et al.9 BFU-E–derived erythroblasts of normal donors cultured from marrow or blood did not differ significantly with respect to pg HbF or percent HbF/BFU-E–derived cells. Similarly, no difference was observed between bone marrow and peripheral blood of American sickle cell patients. Furthermore, repeated studies of the peripheral blood BFU-E HbF program carried out in three Saudi patients and one normal volunteer showed that the standard deviation of the assay performed over a two-week period on a single individual was less than 15%. Therefore, it was concluded that the erythroblasts derived from peripheral blood BFU-E could provide a reliable determination of the HbF program in Saudi patients.

Of note was the fact that the pg HbF and % HbF/ BFU-E–derived cell were statistically different for BFU-E from the peripheral blood of normal donors, American sickle cell patients, and Saudi sickle cell patients (Table 2).

Influence of the HbF program on peripheral blood fetal hemoglobin in sickle cell disease. The influence of the HbF program on the circulating fetal hemoglobin in these Saudi sickle cell anemia patients is shown in Fig 1. Linear
regression analysis of the percentage of fetal hemoglobin in
BFU-E-derived cells versus the percent of fetal hemoglobin
in peripheral blood provided a significant positive correlation
with an r of 0.65. Therefore, the variance of the intrinsic HbF
program appears to account for nearly 40% (r²) of the
variance of the circulating fetal hemoglobin in these patients.
Other factors, particularly selective survival of F cells, must
contribute to the remaining 60%. To determine whether
alpha thalassemia affected hemoglobin production in vitro,
Saudí sickle cell patients were divided into two groups based
on MCV greater than or less than 78. The HbF programs for
both groups were not significantly different (data not
shown).

Figure 2 shows the relationship of the HbF program in
BFU-E-derived cells to the percent fetal hemoglobin in the
peripheral blood of the AS parents of the Saudi sickle cell
anemia patients. The data demonstrate that no correlation
exists between the HbF program and the circulating HbF in
these individuals. Most of these β^4 heterozygotes were par-
ents of patients with circulating high HbF and, as demon-
strated in Fig 3 (discussed below), their HbF programs did
correlate with those of their offspring. This relationship was
not detected from studies of peripheral blood because nearly
all of them had either normal or only slightly elevated
circulating HbF. Three heterozygotes with high HbF pro-
grams had elevated circulating fetal hemoglobin levels (5% to
7%), and two of these individuals had substantially
elevated, albeit unexplained, reticulocyte counts. These
observations suggest that whereas many Saudi β^4 heterozy-
gotes have high HbF programs and have by these criteria a
form of nondeletion HPFH, the mutation is silent in the
peripheral blood unless the rate of erythropoiesis is either
transiently or chronically increased. This occurs when the
increased HbF program is present in the setting of homozy-
gosity for a β^4 gene.

Relationship of patient to parent hemoglobin F pro-
gram. The quantity of hemoglobin F produced in BFU-
E-derived erythroblasts from Saudi sickle cell anemia
patients, their parents, and normal American controls is
displayed in Table 3. Though Saudi AS parents produce half
as much HbF as their SS offspring, their HbF programs are
significantly higher than normal Americans, demonstrating
their inherent capacity for increased fetal hemoglobin pro-
duction.

Table 2. Assessment of HbF in BFU-E-Derived Erythroblasts

<table>
<thead>
<tr>
<th>Patients Source</th>
<th>Normal Donor</th>
<th>American SS</th>
<th>Saudi SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marrow</td>
<td>Blood</td>
<td>Marrow</td>
</tr>
<tr>
<td>No. cells x 10^6/BFU-E-derived colony</td>
<td>10.4 ± 6.2</td>
<td>12.3 ± 5.7</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>pg HbF/BFU-E-derived cell</td>
<td>1.8 ± 0.52</td>
<td>1.3 ± 0.7</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>pg total Hb/BFU-E-derived cell</td>
<td>18.2 ± 2.9</td>
<td>19.6 ± 6.9</td>
<td>14.9 ± 3.0</td>
</tr>
<tr>
<td>% HbF in BFU-E-derived cells</td>
<td>6.8 ± 3.6</td>
<td>6.1 ± 2.1</td>
<td>21.1 ± 3.9</td>
</tr>
<tr>
<td>No.</td>
<td>10</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

*P < 0.01 when compared to normal donor, blood.
†P < 0.001 when compared to American SS, blood.
‡P < 0.001 when compared to normal donor, blood.
§P < 0.025 when compared to American SS, blood.

BFU-E-derived colony size and radioligand assay of fetal and total hemoglobin per BFU-E-derived cell from cultures of bone marrow and peripheral blood of normal donors and American sickle cell disease patients and from peripheral blood of Saudi Arabian sickle cell disease patients.

Fig 1. Percent HbF in peripheral blood versus percent HbF in BFU-E-derived cells in 27 Saudi patients (D) with sickle cell disease. Mean ± 2 SD for normal American donors is shown in the shaded area. r = 0.65, P < 0.05.

Fig 2. Percent HbF in peripheral blood versus percent HbF in BFU-E-derived cells for 27 Saudi patients (D) with sickle cell trait. Mean ± 2 SD for normal American donors is shown in the boxed area.
measured by alkali denaturation. Normal range in less than 2%. The % HbF in BFU-E-derived cells was measured by radioimmunoassay. Measurements indicate mean ± 1 SD.

*P < 0.001 when compared to Saudi AS.
†P < 0.001 when compared to American normal.

Table 3. Assessment of HbF in Saudi BFU-E-Derived Erythroblasts

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No.</th>
<th>% HbF in P.B.</th>
<th>% HbF in BFU-E-Derived Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saudi SS</td>
<td>17</td>
<td>17.4 ± 9.3</td>
<td>30.4 ± 8.6†</td>
</tr>
<tr>
<td>Saudi AS</td>
<td>27</td>
<td>1.2 ± 1.07</td>
<td>16.1 ± 6.4†</td>
</tr>
<tr>
<td>American Normal</td>
<td>8</td>
<td>&lt;2.0</td>
<td>6.1 ± 2.1</td>
</tr>
</tbody>
</table>

The % P.B. HbF represents the percent of HbF in peripheral blood measured by alkali denaturation. Normal range in CHMC hematology lab less than 2%. The % HbF in BFU-E-derived cells was measured by radioimmunoassay. Measurements indicate mean ± 1 SD.

Characterization of the β-globin gene complex by DNA analysis. Five DNA restriction site polymorphisms in the 5' fl globin gene and 3' to it. In several patients, all of the five parental HbF program revealed a significant (P < 0.05) positive correlation (r = 0.51).

In every case, at least one of their parents had an HbF program well above the normal range. Analysis of patient versus mean parental HbF program revealed a significant (P < 0.05) positive correlation (r = 0.51).

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<table>
<thead>
<tr>
<th>Genotype</th>
<th>% HbF in PB.</th>
<th>% HbF in BFU-E-Derived Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saudi AS</td>
<td>17.4 ± 9.3</td>
<td>30.4 ± 8.6†</td>
</tr>
<tr>
<td>Saudi SS</td>
<td>1.2 ± 1.07</td>
<td>16.1 ± 6.4†</td>
</tr>
<tr>
<td>American</td>
<td>&lt;2.0</td>
<td>6.1 ± 2.1</td>
</tr>
</tbody>
</table>

The % PB. HbF represents the percent of HbF in peripheral blood measured by alkali denaturation. The HbF program unless fl is associated with at least one Hind III sites in gamma regions was considered to bear the entire ++ + + + haplotype when the other sites were unknown. Two high HbF patients were heterozygous for the haplotype.

Material suitable for assay of restriction enzyme polymorphisms was available from only one of the three patients with low fetal hemoglobin. Of great interest was the fact that this patient was homozygous for the haplotype – + + + + rather than the common + + + + + seen in the patients with high HbF programs.

To evaluate further the association of the + + + + + haplotype with the HbF program, we analyzed the latter in individuals both homozygous and heterozygous for the haplotype and with different beta-globin genotypes. The results are shown in Table 4. Nearly all individuals either homozygous or heterozygous for the + + + + + haplotype and homozygous for the β gene have markedly elevated HbF programs. That homozygosity for β has no influence on the HbF program unless β is associated with at least one chromosome with the + + + + + haplotype is suggested by the single relevant case. Homozygosity for the + + + + + haplotype does not itself control the level of the HbF program expressed in BFU-E-derived erythroblasts. This is demonstrated by the fact that individuals homozygous for the + + + + + haplotype but heterozygous for β had only modest elevations of HbF programs. Further studies will be required to explain this complicated relationship.

DISCUSSION

Sickle cell anemia patients from the eastern province of Saudi Arabia are unusual because of their high fetal hemoglobin levels, the relative mildness of their clinical disease, and the fact that heterozygotes do not ordinarily exhibit high fetal hemoglobin levels.1-3,17,18 This distinguishes them from individuals heterozygous for the usual deletion or nondeletion forms of hereditary persistence of fetal hemoglobin and renders analysis of the inheritance of the defect difficult to perform.

In an effort to determine whether the high circulating fetal

Table 4. Effect of Interaction of β With the + + + + + Haplotype on the HbF Program

<table>
<thead>
<tr>
<th>B Globin Genotype</th>
<th>% HbF in PB.</th>
<th>% HbF in BFU-E-Derived Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS Homozygous</td>
<td>19.1 ± 8.4</td>
<td>33.0 ± 7.3</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>17.9 ± 28.5</td>
<td>32.4 ± 40.2</td>
</tr>
<tr>
<td>Null</td>
<td>1.7 ± 1.4</td>
<td>17.3 ± 3.6</td>
</tr>
<tr>
<td>AS Homozygous</td>
<td>17.1 ± 1.5</td>
<td>15.5 ± 4.6</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>6.7 ± 1.5</td>
<td>13.0</td>
</tr>
<tr>
<td>Null</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

SS or sickle trait patients are grouped based on being homozygous, heterozygous, or null for the “++ + + +” haplotype in the x through γβ1-globin gene region of the β-globin gene cluster. Due to the strong association of the + Hind III combination with the + + + + + haplotype, any chromosome with the + + Hind III sites in gamma regions was considered to bear the entire + + + + + haplotype when other sites were unknown. The % P.B. HbF represents the percent of HbF in peripheral blood measured by alkali denaturation. The HbF program is represented by % HbF in BFU-E-derived erythroblasts. Results are expressed as mean ± 1 SD.
hemoglobin observed in Saudis with sickle cell anemia is an inherited characteristic, we measured the hemoglobins produced in erythroblasts derived from erythroid progenitors in the circulating blood of the Saudi patients, and their heterozygous parents. This method gives insight into the potential for HbF production in a population of progenitor-derived cells. This potential represents the product of the two independent mechanisms that regulate the circulating fetal hemoglobin level: the HbF-produced per F cell and the number of F cells produced per progenitor cell. The latter cannot be accurately measured due to assay insensitivity that prevents detection of HbF in single cells unless it is present to an extent greater than 3 pg of F per cell (G. Dover, personal communication). Therefore, for the purposes of this assay, we assume that all of the erythroblasts found in progenitor-derived erythroblasts produced in vitro are F cells. The HbF program estimated by this in vitro assay is of total F production, without loss of any population due to selective survival and without admixture of this erythroblast population with cells removed by multiple asymmetric divisions from their progenitors. This is important because present data strongly suggest that the HbF program, while clearly expressed in erythroblasts proximally derived from progenitors, is progressively diminished as erythroblasts pass through progressive rounds of asymmetric mitosis.

Saudi sickle cell anemia patients produce increased levels of fetal hemoglobin in culture, compared to normal American controls. The variance of the progenitor-regulated HbF program partially explains the variance of the circulating fetal hemoglobin of Saudi sickle cell patients but other factors including selective survival of erythrocytes containing fetal hemoglobin have a significant role. That the increased HbF program of Saudi sickle cell anemia patients is highly variable suggests that the gene is highly regulated. In this respect, it differs from many of the known causes of HPFH in which the levels of HbF in peripheral blood of heterozygotes is quite constant. Indeed, the only constant finding with respect to the elevation of HbF in these patients is the regularly detected increased ratio of Gα to Aα globin. In the Saudi patients, Gγ represents 76.5 ± 5.1% of total HbF. In contrast, the Gγ to Aγ ratio in sickle cell anemia patients of African origin is variable but usually low.

An important aspect of the data presented here concerns the detection of a variant of nondeletion HPFH in at least half of the β^+^ heterozygous parents of Saudi sickle cell patients with high circulating fetal hemoglobin. An increased level of circulating HbF is in most cases barely detectable in them, though the potential for high HbF production is found in the progenitors of many. The only heterozygotes with increased circulating fetal hemoglobin also had reticulocytosis. We hypothesize, therefore, that expression of the high HbF program may require erythropoietic stress such as the sickle cell anemia present in their children. The increased erythropoietin secretion secondary to anemia rapidly forces the generation of erythroblasts directly from relatively immature progenitor cells, many of which produce erythroblasts with elevated HbF expression. This model predicts that the actual amount of HbF produced in the erythroblasts derived from these progenitors depends upon two factors; the state of maturation of the progenitors (perhaps with respect to the number of quantal mitoses completed in vivo prior to the culture) and the level of permissiveness of the gamma-globin gene.

Rank order analysis of patients and parental HbF programs shown in Fig 3 demonstrate an important relationship. Above an HbF program of 2.2 pg per cell, there was a significant correlation between the HbF program detected in the progenitors of the patients with that of at least one of their parents. These data provide strong evidence that the high fetal hemoglobin in Saudi sickle cell disease is inherited. These data plus those presented in Table 3 suggest but do not prove that, in Saudis, the high HbF program may be linked to the beta-globin gene cluster of chromosome 11.

To begin to examine the molecular basis of the increased HbF program in Saudis, we ascertained the haplotype of the epsilon through ψβ1 region of chromosome 11 using restriction enzyme site polymorphisms. The limited haplotype data demonstrated that the ψ′ + + + + + 5' haplotype is extremely common in these Saudis with sickle cell anemia and high fetal hemoglobin. This haplotype has been associated with Swiss HPFH,13 and when linked to β^+^ has been associated with markedly raised fetal hemoglobin levels in Saudi Arabians13, and in a Jamaican family of mixed Asian Indian/African ancestry.14 The haplotype “− + + + + + ” is one of the most common haplotypes on normal chromosomes.23 Common to the two haplotypes are the four 3' sites “− + + +” and, where studied, atypically high levels of Gγ. Also associated with some samples with this haplotype is a thymine for cytosine substitution at position −158 5' to the Gγ gene,39 which could itself increase the efficiency of the Gγ promoter, or merely represent an irrelevant but linked DNA polymorphism. Even if the −158 C → T substitution or another mutation in the + + + + + + haplotype is responsible for increased Gγ/Aγ ratio, another regulatory system, perhaps akin to the recently reported −202 mutation 5' to Gγ30 or even separable from the β-globin gene cluster31 may be responsible for the high total HbF production. The Saudi mutation is probably different from the −202 HPFH mutation because it appears to require anemic stress for expression. Cloning and sequencing of the γ gene flanking regions is necessary to determine all regions of difference between haplotypes. Systems for the study of the fetal to adult globin switch will also be necessary for full understanding of the relevance of point mutations detected.

Finally, though sickle cell disease in the eastern province of Saudi Arabia appears to be mild, and the combination of HPFH with sickle cell disease thought to ameliorate the latter,22,23 the elevated HbF that characterizes these patients may not be solely responsible for their more benign clinical status. G6PD deficiency is common. Almost half of this population has microcytosis due to alpha thalassemia.29 Alpha thalassemia is unlikely to be responsible for the elevated HbF in Saudis since patients with alpha thalassemia and sickle cell disease have lower levels of HbF than nonthalassemic sickle cell patients.44 Although neither alpha thalassemia nor increased HbF, themselves, necessarily reduce the
clinical severity of sickle cell disease.\textsuperscript{35,36} the combination of both may be important. Only a field study will clarify that important issue. Clearly, continued studies of sickle cell anemia in the eastern province of Saudi Arabia will provide important information about the molecular biology of fetal hemoglobin production and the interacting factors affecting clinical severity.

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