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Hb F in sickle cell anemia

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Abstract. We have reviewed the methodology for an accurate quantitation of Hb F in the blood of patients with sickle cell anemia, values observed in hundreds of patients of different (racial or ethnic) backgrounds and with differences in severity of the disease, and the various factors that affect the level of Hb F. The latter include sex, age, genetic background or chromosomal haplotypes, variations in the sequences of the locus control region(s) 5′ to the ε-globin gene, and the presence of an α chain deficiency or α-thalassemia. Finally, a few remarks about agents effective in increasing the in vivo Hb F synthesis are also included.

Key words. γ-Globin genes; haplotypes; α-thalassemia; locus control region; clinical expression; hydroxyurea; erythropoietin.

Introduction

Human fetal hemoglobin or Hb F consists of two α and two γ chains ($\alpha_2\gamma_2$) and is the major Hb type of the fetus and the newborn. The synthesis of the α chains is directed by two α-globin genes (α1 and α2); the α2-globin gene is the major type but its exons are identical to those of the α1-globin gene and thus only one type of α chain is synthesized[42,55]. The γ-globin gene is also duplicated; the $^{G}\gamma$ chain has a glycine residue at position 136 and the $^{A}\gamma$ chain has an alanine residue at that location (codon 136 $^{G}\gamma$:GGA; codon 136 $^{A}\gamma$:GCA)[83]. The level of Hb F in a normal newborn baby varies between 70 and 85% with a $^{G}\gamma$ value of about 70% and $^{A}\gamma$ of about 30%[46,83]. After birth the Hb F level decreases rapidly to 1–2% at the age of 6 months; the $^{G}\gamma$ to $^{A}\gamma$ ratio also decreases in the majority of infants from 70:30 to 40:60[46,82].

Babies with sickle cell anemia (SS) are born with a high level of Hb F (85–95%) and its decrease after birth is considerably slower than seen in normal infants[96]. Furthermore, SS patients 1 year and older have increased levels of Hb F which vary considerably but appear rather constant in each patient after the age of 5–7 years. Figure 1, taken from a study conducted during the 1960's and early 70's[96], shows that the Hb F in Black adult SS patients varies from 2 to 20%, while the Hb F level changes only slightly when measured in several SS individuals repeatedly over a period of years. The purpose of this paper is to summarize methodology most useful for an accurate determination of the level of

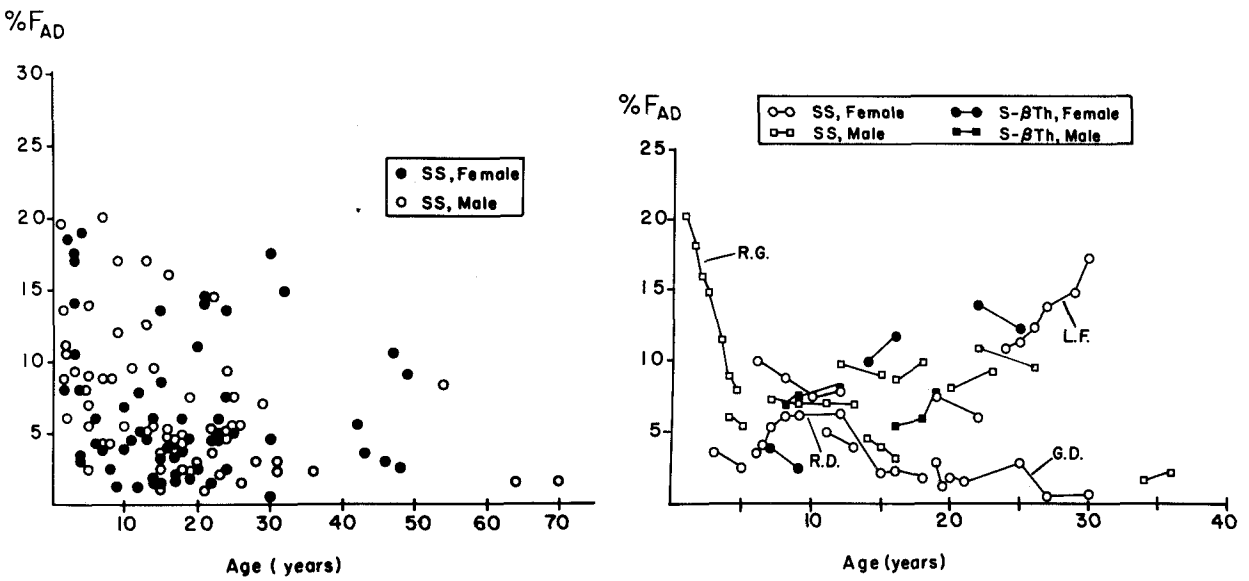


Figure 1. *Left.* The levels of Hb F in SS patients of different ages. *Right.* The levels of Hb F in several SS patients and in patients with Hb S-β-thal, determined successively over the years. Hb F was quantified with the method of Betke et al.[13]. The data are from Wrightstone and Huisman[96].

Hb F; to evaluate the role of Hb F in modulating the clinical course of SS disease and its interaction with Hb S in the sickling process; to review the various genetic determinants of the β-globin gene cluster involved in the expression of γ-globin genes; to determine the influence of age and sex on the Hb F level and the $^{G}\gamma/^{A}\gamma$ ratios, and finally, to review possible mechanisms of action of pharmacological agents stimulating Hb F production.

Methods of determining the Hb F, $^{G}\gamma$, and $^{A}\gamma$ levels

Hb F

Older methods have been reviewed in a previous monograph[45]. One of these, the alkali denaturation procedure developed by Betke et al.[13] is still most popular and relatively simple. Its disadvantage is that low values ($<5\%$ Hb F) are determined too high and high values ($>20\%$) too low, while percentages between 5 and 20% can be obtained with a reasonable accuracy[50]. The method should probably be replaced by chromatographic procedures using cation exchangers[81]. A simple micro-method that is suitable for blood samples from SS and S-β°-thalassemia (thal) patients who have not been transfused, has been described[1] and is commercially available (Isolab, Inc., Akron, OH, USA). This technique cannot be used for samples containing Hb A because the minor Hb A_1 component elutes with Hb F. Perhaps most suitable are two recently developed high performance liquid chromatography (HPLC) procedures (fig. 2). The first uses a cation exchange material[14,50] and allows the complete separation of Hb F (as Hb F_0 and Hb F_1) from other Hb types in less than 50 min. The second is a reversed phase HPLC method with a 4.6×250 mm C_4 column separating heme and the various globin chains[51,87].

The first method used to evaluate the distribution of Hb F among red cells was developed by Kleihauer et al.[48]. Although this procedure is still in use for that purpose, it is replaced by one that uses monospecific anti F antibodies[15,23]. Besides detection of Hb F the method makes it possible to quantitate the Hb F level/cell. Both procedures have shown that Hb F is heterogeneously distributed in the red cells (fig. 3). Normal adults have their Hb F confined to a subpopulation of F cells that comprises 0.1–0.7% of the total number of red blood cells (RBCs)[15]. In most cases of hemolytic anemia slightly elevated levels of Hb F and elevated numbers of F cells are observed. The overall production of Hb F and F cells is considerably increased in SS patients, while a preferential survival of Hb F-containing cells further augments these numbers[24]. However, in pancellular hereditary persistence of Hb F (HPFH), there is an increased production of Hb F which is homogeneously distributed among the RBCs[46]. This condition is mainly present in Blacks; it has been estimated to occur in 0.1–0.2% of the Black population of the United States and Jamaica. The compound heterozygous (S-HPFH) condition is detected once in every 11,000 births in Jamaica, while American data suggest a prevalence of 1 in 25,000 births.

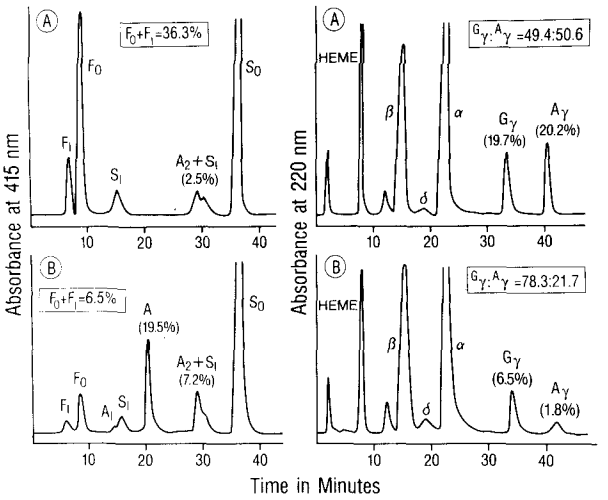


Figure 2. Quantitation of Hb F by HPLC procedures. *A* A young SS patient with a high level of Hb F that was estimated at 36.3% ($=F_0+F_1$) by cation exchange HPLC[14,50] and at 39.9% ($=^{G}\gamma+^{A}\gamma$ as % of $\beta+^{G}\gamma+^{A}\gamma$) by reversed phase HPLC. *B* Similar data for an older patient with Hb S-β°-thal. The Hb F level was 6.5% (F_0+F_1) by cation exchange HPLC and 7.3% ($^{G}\gamma$ and $^{A}\gamma$) by reversed phase HPLC. It is not unusual to find slightly higher Hb F levels by reversed phase HPLC (as % $^{G}\gamma$ and $^{A}\gamma$) perhaps because of a slight contamination of $^{G}\gamma$ with α. Hb A_2 separates only partially from a minor Hb S_1 zone by the cation exchange HPLC procedures; the separation can be greatly improved with the use of a considerably slower gradient. The ratio between the $^{G}\gamma$ and $^{A}\gamma$ chains can readily be obtained with the reversed phase HPLC procedure; the nearly identical level of $^{G}\gamma$ and $^{A}\gamma$ in case *A* suggests that this young SS patient is in the process of changing the newborn $^{G}\gamma$ to $^{A}\gamma$ ratio (70:30) to the so-called adult ratio (40:60). The high $^{G}\gamma$ level in case *B* is not surprising because the two most common Black β-thal mutations are associated with high $^{G}\gamma$ values[37].

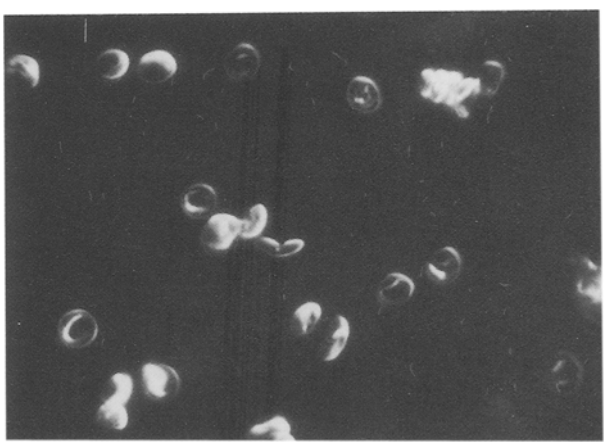


Figure 3. Detection of fetal Hb inside red cells with a fluorescent anti Hb F antibody (courtesy of Dr George J. Dover, Baltimore, MD, USA).

The relative quantities of the $^{G}\gamma$ and $^{A}\gamma$ chains in Hb F

Different chemical, chromatographic, and electrophoretic methods have been developed (reviewed in refs 44 and 46). All these have been replaced by the reversed phase HPLC method developed by Shelton et al.[87] and further modified by Kutlar et al.[51]. A complete separation of the $^{G}\gamma$ and $^{A}\gamma$ chains can be obtained (fig. 2) and calculation of their relative quantities can readily be made. The frequently occurring $^{A}\gamma^{T}$ chain, a variant of the $^{A}\gamma$ chain because of an Ile → Thr replacement at position $^{A}\gamma$ 75[78], elutes between the α and $^{G}\gamma$ chains and can readily be quantitated with the method. When the Hb F level is less than 10% it is advisable to isolate the Hb F prior to the chain analysis; a simple DEAE-cellulose chromatographic method[81] using a 1 × 15 cm column is adequate for that purpose.

Interaction of Hb S and Hb F in sickling

There is well-documented information about the interaction of Hb S with other Hb types (including Hb F) in gelation within the red cell that leads to the sickling process[8,10,17,19,36,53,61]. A mixture of Hb S and Hb F (as deoxy-Hb) has a high solubility and it is likely that the formation of an asymmetrical hybrid ($\alpha_2\gamma\beta^S$) is the primary cause of the inhibition of polymerization by Hb F. The mild expression of the Hb S-HPFH condition with about 70% Hb S and 30% Hb F is due to the homogeneous distribution of Hb F within the red cells; the formation of the soluble hybrid ($\alpha_2\gamma\beta^S$) in all red cells protects these from sickling.

Hofrichter et al.[43] studied the kinetics of gelation and observed that mixtures of Hb S and Hb A or Hb F (as deoxy-Hb) showed a prolongation of the time prior to polymerization when compared to pure Hb S; the effect was considerably more marked with Hb F than with Hb A. It was suggested that this prolonged delay time might prevent sickling from occurring in vivo, because it is sufficiently long for the RBCs to return to the lungs to be reoxygenated, or at least to escape from the microvasculature prior to polymerization. Using data from patients with Hb Sβ^+-thal (15–30% Hb A), Hb S-HPFH (20–30% Hb F), and sickle cell trait (60% Hb A), Sunshine et al.[92] predicted the changes in delay time that would have to be produced by a therapeutic agent to result in less severe disease, much less severe disease, or no disease.

Small variations in MCHC have a marked effect on the delay time as is evidenced by the irreversibly sickled cells (ISCs) that have high MCHC values and sickle readily. Bertles and Milner[12] demonstrated that the synthesis of Hb F is low in RBCs that eventually become ISCs, thus implying that Hb F in the non-ISCs protected these cells from the process or processes that produce the irreversibly sickled forms (see also refs 57 and 86). The pathophysiology of SS disease appears to be significantly influenced by the dense fraction of erythrocytes that can be obtained by density gradient centrifugation[30,47]. This technique reveals marked erythrocyte heterogeneity; the proportion of the most dense cells correlates with the percent ISCs present. These cells have an increased mechanical fragility and hemolyze readily when subjected to physiologic shear forces. The rheologic properties of dense cells appear to be directly related to their MCHC and Hb F levels.

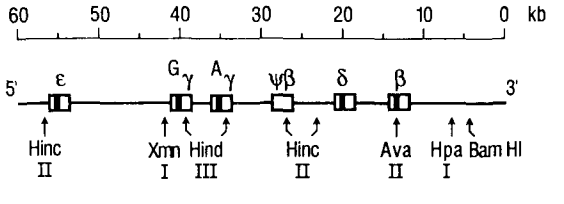
Relationship between Hb F and clinical severity

Serjeant[85] found splenomegaly to be more common in SS patients with Hb F levels above 8% and suggested that the difference from patients with lower Hb F levels reflected a lesser tendency to infarction. Stevens et al.[91] studied the same group of patients and found that low Hb F levels in patients 6 months of age was predictive of those who would later develop early splenomegaly, hand and foot syndrome, or splenic sequestration, as well as those who would die before the age of 2 years. Powars et al.[75] evaluated a group of SS patients aged from less than 10 years to more than 40 years and concluded that there was an inverse relationship between a high level of Hb F and the occurrence of stroke, but not between the Hb F level and painful crisis, acute chest syndrome, aseptic necrosis, meningitis, septicemia or death. In a later study[74], after following patients from birth to 56 years for an average of 11 years, it was concluded that strokes and aseptic necrosis were less common if the Hb F level was more than 10%, and crises and pulmonary disorders were less common if the Hb F level was more than 20%. Rucknagel et al.[79] found that hospitalizations and transfusions but not pain crises were fewer in children with high Hb F levels. Similarly, Odenheimer et al.[67] found that the Hb F level was a strong predictor of a patient's hospitalization and transfusion status, but a poor predictor of pain crises.

Genetic determinants of γ-globin gene expression

Haplotypes

There is convincing evidence that the β^S mutation arose in Africa and Saudi Arabia-India on at least five different types of chromosomes with distinct haplotypes, linked to the β^S-globin gene and numbered 3, 17, 19, 20, and 31 or labeled Senegal, Cameroon, Benin, Bantu or Central African Republic (CAR), and Saudi Arabia-India types, respectively[5,40,62,63,84] (fig. 4). There are differences in the clinical course of SS patients who carry the different haplotypes; patients with haplotypes No. 3 and No. 31 have higher levels of Hb F and a milder disease. The presence of one specific haplotype does not, however, appear to be the only cause of increased Hb F levels because there is considerable variation within each group (see below).



Haplotype	1	2	3	4	5	6	7	8	9
Saudi Arabian/Indian (#31)	+	+	+	−	+	+	+	+	−
Senegal (#3)	−	+	+	−	+	+	+	+	+
Benin (#19)	−	−	−	−	−	+	+	−	+
Bantu (#20)	−	−	+	−	−	−	+	+	+
Cameroon (#17)	−	−	+	+	−	+	+		+

Figure 4. The five major haplotypes of the β^S chromosomes. The number of restriction sites is usually limited to the nine listed here.

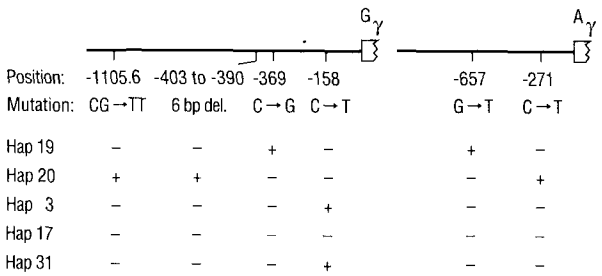


Figure 5. Specific differences in the sequences of the 5′ flanking regions of the $^G\gamma$- and $^A\gamma$-globin gene promoter regions of β^S chromosomes with specific haplotypes. Numbering of the haplotypes is as in refs 5 and 40 (modified from Dimovski et al.[22]). Haplotype No. 17 has a T→C mutation in condon 75 of the $^A\gamma$-globin gene resulting in the synthesis of the $^A\gamma^T$ chain (75 $^A\gamma$ Ile→Thr; Ricco et al.[78]).

Several investigators proposed the possible presence of sequences within the β-globin gene cluster that may act as enhancers or silencers of γ-globin gene expression[11,28,64]. A search for these has prompted sequence studies of the two γ-globin genes and their flanking regions. Ragusa et al.[77] documented four sequence variations (C→T at −158 5′ to $^G\gamma$, T→C at +2285, C→A at +2476, and A→G at +2676 3′ to $^A\gamma$) in a Sicilian patient with a nondeletional HPFH who was homozygous for haplotype No. 3. However, the same set of mutations was found in an Algerian patient with severe β^0-thal who was also homozygous for haplotype No. 3 but had very low levels of Hb F. It appears, therefore, that these variations do not play a major role in the regulation of Hb F production.

Economou et al.[26] studied single nucleotide substitutions in the promoter regions 5′ to the $^G\gamma$- and $^A\gamma$-globin genes in SS patients with different haplotypes and varying levels of Hb F. They examined the promoter regions from −350 bp to +50 bp relative to the Cap site. No mutations were found in either region suggesting that nucleotides in these regions are not responsible for the marked variation in Hb F production among SS individuals.

Lanclos et al.[54] and Dimovski et al.[22], however, detected certain mutations within the promoter regions of the $^G\gamma$- and $^A\gamma$-globin genes and in the second intervening sequence (IVS-II) of the $^A\gamma$-globin gene that are characteristic for the different haplotypes (fig. 5). A β^S chromosome with haplotype No. 19 has two specific mutations, one in each of the promoter sequences, while that with haplotype No. 17 has none of these but carries a T→C mutation at codon 75 ($^A\gamma$) leading to the synthesis of the $^A\gamma^T$ chain (75 Ile→Thr). A β^S chromosome with haplotype No. 20 has two mutations and one 6 bp deletion in the $^G\gamma$ promoter and one mutation in the $^A\gamma$ promoter. β^S chromosomes with either haplotypes No. 3 or No. 31 are indistinguishable from each other but both have the C→T mutation at −158 in the $^G\gamma$ promoter.

The C→T mutation at −158 (Xmn I site)

Several studies[7,33,34,41,49,52,58,59] have shown that a C→T replacement at position −158 5′ to the $^G\gamma$-globin gene (that creates a new Xmn I site) is associated with high $^G\gamma$ and higher Hb F levels. Miller et al.[58,59] demonstrated a positive Xmn I site in almost 100% of Saudi Arabian SS and AS individuals and in 22% of AA individuals from that country. However, homozygosity for this mutation had no major effect on Hb F production in the normal (AS and AA) Saudi population. The same observation has been made for other populations[27]. While this mutation may not be uniquely responsible for high levels of Hb F, it may have an important role in regulating its production and requires interaction with additional factors such as hemolytic stress or other molecular determinants, possibly linked to the β^S gene[39].

Hattori et al.[40] were the first to study the relationship between haplotypes, hematological indices, Hb F and $^G\gamma$ values in Black American patients. These studies and those by others[52,64] indicated that the presence of at least one chromosome with haplotype No. 3 (Senegal) is sufficient to improve hematological data and to increase the $^G\gamma$ and Hb F values. The large variability in $^G\gamma$ and Hb F values again suggests that other genetic determinants are involved, including genes not directly linked to the β-globin gene cluster. Ballas et al.[7] extended the studies mainly to haplotype No. 3 homozygotes and demonstrated a significant correlation between the presence of the Xmn I site and an increased $^G\gamma/^A\gamma$ ratio in a dose-dependent manner.

The locus control region (LCR) and Hb switching

Synthesis of the embryonic, fetal, and adult Hbs are activated at different stages in human development[89,90]. Expression of the different Hb types is under the direct control of the appropriate globin gene, but activation of the β-globin gene domain that facilitates globin gene

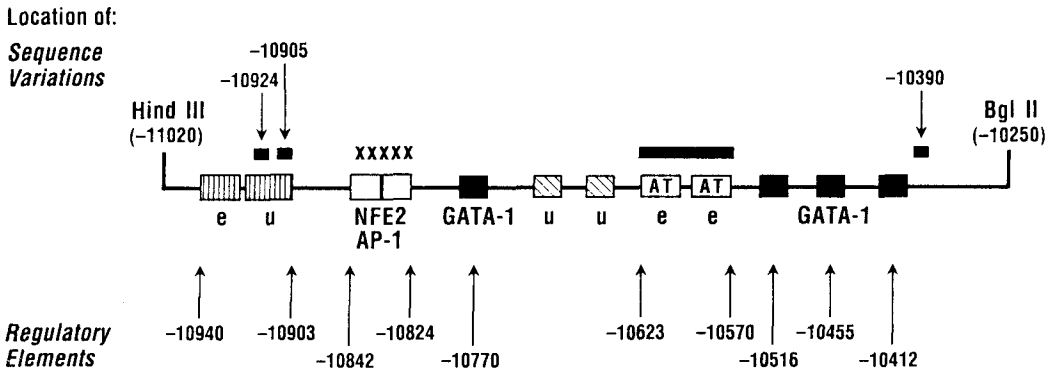


Figure 6. Comparison of sequence variations in the 5'HS-2 of a β^S chromosome with haplotype No. 19 with known DNase I footprinting sites. The diagram is not to scale. The areas designated by e are footprints that appear only in erythroid extracts, u indicates ubiquitous footprints, and AT represents the presence of AT repeat sequences. The solid bars denote the regions of sequence variations in haplotype No. 19. The xxxxx shows the location of the region with maximal enhancer activity contained with tandem AP-1 binding sites. The numbers indicate positions relative to the Cap site of the γ-globin gene (from Öner et al.[69]).

transcription in erythroid cells, is largely regulated by the LCR[94]. This LCR was first described as a cluster of five deoxyribonuclease (DNase) I hypersensitive (HS) sites; four sites (HS-I-IV) are located 6–18 kb upstream of the ε-globin gene, and a fifth (HS-V) is located 20 kb downstream from the β-globin gene. These HS sites confer both temporal and tissue specificity of gene expression by organizing the entire β-globin locus into an open domain, thus conferring erythroid specific expression on the globin genes. They also act as enhancers of globin gene transcription[31]. Studies in transgenic mice and transfected erythroid cells have shown that the LCR allows for a high level of position-independent, copy-number-dependent, erythroid-specific expression[9,38,76,88,95]. One segment (HS-2) of the LCR alone can confer a high level of expression in the range of about 40% of the level obtained with the complete LCR[72]. This HS-2 appears to contain several conserved sequences that bind erythroid as well as ubiquitous nuclear transcriptional factors, while alterations in some of these sequences have dramatically changed the transcriptional enhancing activity of HS-2 in both transgenic mice and cultured erythroid cells[16,21,29,32,56,65,72,80,93]. The locations of the sequence variations and regulatory elements are depicted in figure 6.

Hb F and $^G\gamma$ levels among SS patients of various nationalities and β^S haplotypes

Table 1 summarizes data obtained for 249 Nigerian, 51 Turkish, and 42 American SS patients with a homozygosity for haplotype No. 19. The table also contains values for 63 SS patients from Kenya and Tanzania homozygous for haplotype No. 20, and 40 Indian SS patients homozygous for haplotype No. 31. Figure 7 shows the distribution of Hb F and $^G\gamma$ levels in all patients according to their haplotypes. The $^G\gamma$ levels followed a normal distribution, while the distribution of Hb F was skewed to the left in the patients with haplotypes Nos. 19 and 20, and to the right in those with haplotype No. 31. As expected, the homozygous haplotype No. 31 patients had the highest mean values of Hb F and $^G\gamma$. The Eti-Turks, among the patients with homozygous haplotype No. 19, had significantly higher Hb F levels, but the mean $^G\gamma$ levels were all within a narrow range of 37–42%.

Influence of age and sex on Hb F and $^G\gamma$ levels

Hb F levels are generally higher in SS patients below the age of 5 years and fall sequentially thereafter (table 2). Within the three major haplotypes, the mean Hb F

Table 1. Hematological data for SS patients with different β^S haplotypes[a]

Haplotype	n	Age years	Hb g/dl	RBC 10^{12}/l	MCH pg	Hb A_2 %	Hb F %	$^G\gamma$ %
19/19[b]	342	9.9 ± 6.0	7.6 ± 1.3	2.65 ± 0.63	29.2 ± 5.1	3.0 ± 1.1	8.9 ± 5.6	41.6 ± 6.3
20/20[c]	63	10.6 ± 6.0	7.5 ± 1.3	2.3 ± 0.5	32.9 ± 7.0	3.3 ± 1.5	8.0 ± 5.5	41.2 ± 5.4
31/31[d]	40	12.0 ± 9.9	9.7 ± 2.2	3.16 ± 1.02	31.9 ± 7.8	1.8 ± 0.8	23.3 ± 10.2	68.5 ± 4.3

[a]Average values and SD. [b]Includes combined data for American, Nigerian, and Turkish patients (from refs 2, 4, and 70). [c]Data for Kenyan and Tanzanian patients (from refs 68 and 70). [d]Data for Indian patients (from ref. 39).

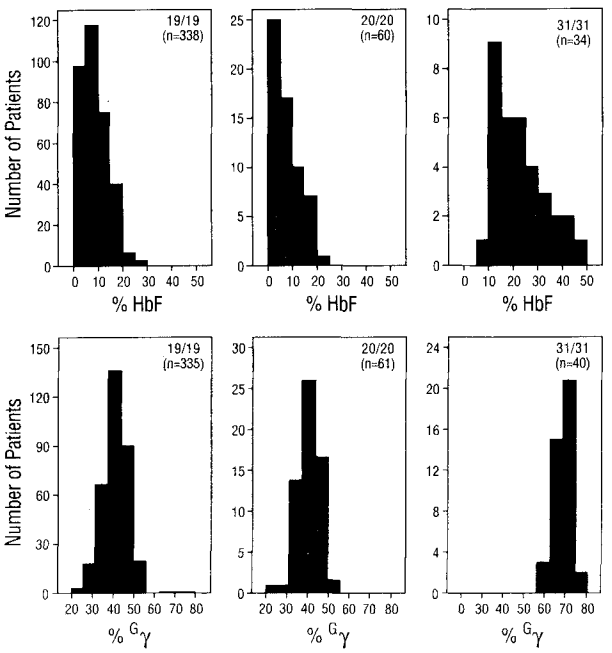


Figure 7. Distribution of the Hb F and $^{G}\gamma$ percentages among SS patients with homozygosities for haplotypes Nos. 19, 20, and 31.

levels are somewhat higher among female than male patients, although the difference often does not reach statistical significance (table 3). Nagel and Ranney[63] have suggested that a factor linked to the X chromosome influences the level of Hb F both in normal individuals as well as in SS patients. Females, carriers of two X chromosomes, may be homozygous for this factor and hence express, in a subset, higher levels of Hb F. Morris et al.[60] have also recently shown that Hb F levels in male Jamaican patients show a sequential fall with age, while the levels in female patients are consistently higher in all age groups.

Influence of α-thal on Hb F levels

Noguchi et al.[66] showed that there is no difference in the level of Hb F, the % F reticulocytes, or the % Hb F/cell between SS individuals with two or four α-globin genes. However, Dover et al.[25] demonstrated a strong inverse correlation between non-F cell levels and a decreasing α-globin gene number, and concluded that falling Hb F levels in SS patients with α-thal reflect preferential survival of non-F cells, and are not due to intrinsic differences in F cell production or in the amount of Hb F/cell. The improved survival of non-F cells was attributed to the lower MCHC observed in such individuals. Moreover, α-thal results in decreased hemolysis in SS patients with consequent increases in Hb and PCV values and lower reticulocyte counts. The data in table 4 and in figure 8 support these conclusions; the effect of a severe α chain deficiency is most striking in SS patients with high levels of Hb F.

Table 2. Influence of age on the hematological data of SS patients with different β^S haplotypes[a]

Haplotype	Age years	n	Hb g/dl	RBC 10^{12}/l	MCH pg	Hb A_2 %	Hb F %	G_γ %
19/19[b]	<5	90	7.5 ± 1.2	2.79 ± 0.65	27.1 ± 4.1	2.9 ± 1.0	10.1 ± 5.9	42.4 ± 5.3
	6–10	119	7.5 ± 1.2	2.56 ± 0.55	29.8 ± 5.7	3.0 ± 1.0	8.7 ± 5.7	40.5 ± 5.2
	11–15	80	7.6 ± 1.2	2.61 ± 0.58	29.5 ± 4.7	3.0 ± 0.7	8.8 ± 5.5	41.6 ± 7.1
	>15	58	8.1 ± 1.7	2.76 ± 0.79	30.7 ± 4.9	3.4 ± 1.7	7.9 ± 5.1	41.9 ± 7.5
20/20[c]	<5	13	7.5 ± 0.7	2.48 ± 0.43	30.1 ± 4.1	2.8 ± 0.8	9.1 ± 6.5	42.1 ± 6.7
	6–10	22	7.2 ± 1.5	2.20 ± 0.61	33.8 ± 9.2	3.3 ± 1.4	7.4 ± 4.8	40.5 ± 5.3
	11–15	12	7.6 ± 1.0	2.24 ± 0.37	33.7 ± 6.8	3.1 ± 0.6	7.5 ± 5.8	41.1 ± 4.5
	>15	16	8.0 ± 1.6	2.40 ± 0.46	33.3 ± 5.2	4.0 ± 2.3	7.8 ± 6.1	41.4 ± 5.4
31/31[d]	<5	10	9.0 ± 2.2	3.22 ± 1.15	29.8 ± 7.6	2.1 ± 1.1	23.4 ± 11.7	69.1 ± 11.6
	6–10	14	10.2 ± 2.2	3.51 ± 0.91	29.6 ± 3.4	1.7 ± 0.9	26.9 ± 12.9	67.3 ± 5.0
	11–15	6	9.2 ± 1.8	2.58 ± 0.77	35.6 ± 9.0	1.7 ± 0.5	22.7 ± 8.2	70.1 ± 1.8
	>15	10	9.9 ± 2.6	2.98 ± 0.91	34.9 ± 10.7	1.9 ± 0.7	19.7 ± 6.7	68.5 ± 3.9

[a]Average values and SD; the data are from references 2, 4, 39, 68, and 70. [b]Combined data for American, Nigerian, and Turkish patients. [c]Combined data for Kenyan and Tanzanian patients. [d]Data for Indian patients.

Table 3. Sex distribution of the hematological data of SS patients with different β^S haplotypes[a]

Haplotype	Sex	n	Age years	Hb g/dl	RBC 10^{12}/l	MCH pg	Hb A_2 %	Hb F %	$^{G}\gamma$ %
19/19[b]	Male	168	9.6 ± 6.3	7.6 ± 1.4	2.68 ± 0.61	28.6 ± 4.6	3.2 ± 1.3	8.6 ± 5.8	40.8 ± 5.9
	Female	179	10.2 ± 5.8	7.7 ± 1.3	2.63 ± 0.66	30.0 ± 5.5	2.9 ± 0.9	9.3 ± 5.4	42.1 ± 6.3
20/20[c]	Male	32	10.5 ± 6.7	7.5 ± 1.1	2.39 ± 0.45	31.5 ± 5.8	3.7 ± 1.9	6.8 ± 5.3	40.9 ± 5.4
	Female	31	10.7 ± 5.4	7.5 ± 1.5	2.24 ± 0.55	34.3 ± 7.8	2.9 ± 0.8	9.3 ± 5.5	41.4 ± 5.4
31/31[d]	Male	24	14.5 ± 11.4	9.7 × 2.1	2.99 ± 0.96	34.5 ± 8.6	1.9 ± 0.9	22.2 ± 9.5	68.8 ± 4.1
	Female	16	8.2 ± 5.4	9.7 ± 2.6	3.44 ± 0.95	27.9 ± 3.9	1.8 ± 0.8	25.3 ± 11.5	68.0 ± 4.6

[a]Average values and SD; the data are from references 2, 4, 39, 68, and 70. [b]Combined data for American, Nigerian, and Turkish patients. [c]Combined data for Kenyan and Tanzanian patients. [d]Data for Indian patients.

Table 4. Influence of α gene deletions on the hematological data of SS patients with different β^S haplotypes[a]

Haplotype	Number of α genes	n	Age years	Hb g/dl	RBC 10^{12}/l	MCH pg	Hb A_2 %	Hb F %	$^G\gamma$ %
19/19[b]	4	187	9.8 ± 5.8	7.5 ± 1.3	2.50 ± 0.53	30.5 ± 5.2	2.8 ± 0.9	9.2 ± 5.4	42.1 ± 5.8
	3	99	9.8 ± 5.7	7.7 ± 1.3	2.79 ± 0.58	27.8 ± 4.3	3.3 ± 1.2	8.5 ± 5.7	41.7 ± 6.9
	2	18	12.5 ± 6.6	8.1 ± 1.6	3.68 ± 0.92	21.6 ± 1.8	3.6 ± 1.7	7.5 ± 5.2	38.5 ± 5.7
20/20[c]	4	26	11.5 ± 7.1	7.5 ± 1.4	2.18 ± 0.55	35.2 ± 7.8	2.8 ± 0.7	8.5 ± 6.1	40.8 ± 4.9
	3	2	11.0 ± 5.6	7.5 ± 1.2	2.44 ± 0.45	30.9 ± 5.5	3.9 ± 1.9	6.5 ± 4.1	40.9 ± 5.8
	2	1	8.0	7.1	2.9	24.1	4.2	5.5	39.3
31/31[d]	4	8	14.6 ± 11.7	9.7 ± 2.8	2.95 ± 0.97	33.6 ± 5.9	1.5 ± 0.8	23.5 ± 9.9	68.2 ± 5.5
	3	19	9.9 ± 6.6	10.1 ± 2.0	3.39 ± 0.98	30.7 ± 7.4	1.6 ± 0.7	27.4 ± 11.3	69.2 ± 3.6
	2	10	11.6 ± 6.2	8.7 ± 2.0	2.96 ± 0.96	30.8 ± 6.8	2.6 ± 0.8	16.0 ± 3.9	68.3 ± 4.7

[a]Average values and SD; the data are from references 3, 4, 39, 68, and 70. [b]Combined data for American, Nigerian, and Turkish patients. [c]Combined data for Kenyan and Tanzanian patients. [d]Data for Indian patients.

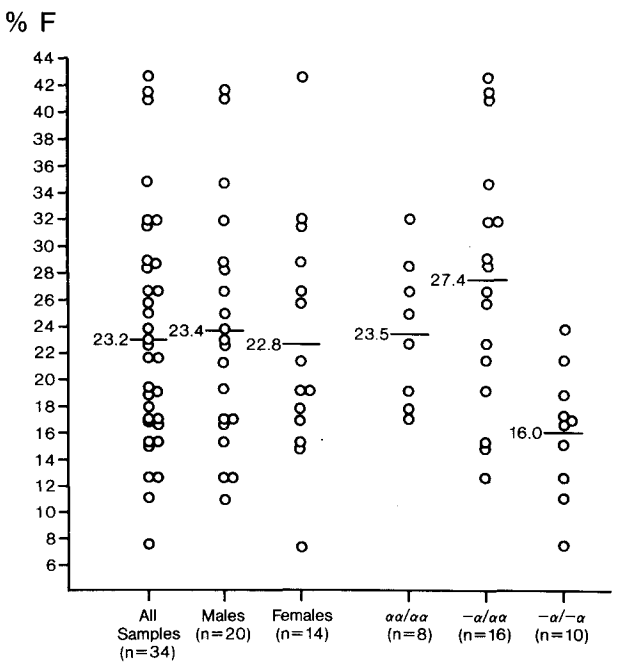


Figure 8. Hb F levels in 34 patients with a homozygosity for haplotype No. 31 and a comparison between males and females, and between patients with different numbers of α-globin genes (from Gupta et al.[39]).

The effect of mutations in the LCR region (LCR HS-2)

Öner et al.[69] studied sequence variations in the HS-2 from β^S chromosomes of SS patients homozygous for haplotype No. 19 (low Hb F) and haplotype No. 3 (high Hb F), and from a β^S chromosome with haplotype No. 19 exhibiting high γ chain expression and high $^G\gamma$ levels. Several nucleotide variations were present in the HS-2 of the haplotype No. 19 individual (fig. 9). One of these is the A → G mutation at position −10905 that creates an Sp1 binding site. Dot-blot analysis demonstrated that these variations are specific for β^S chromosomes with haplotype No. 19. In one SS patient who was homozygous for haplotype No. 19 but had high $^G\gamma$ and high Hb F values, a crossover was observed that placed sequences similar to the HS-2 of haplotype No. 3 in juxtaposition to the 5′ flanking regions of haplotype No. 19. It was, therefore, concluded that a β^S chromosome with haplotype No. 19 but having a HS-2 characteristic for haplotype No. 3 is associated with high γ chain expression. The sequence data for this atypical haplotype No. 19 indicated that the C → G mutation at position −369 5′ to $^G\gamma$, that is characteristic of haplotype No. 19, was absent, while the A → G mutation at position −309 5′ to $^G\gamma$ and the G → T

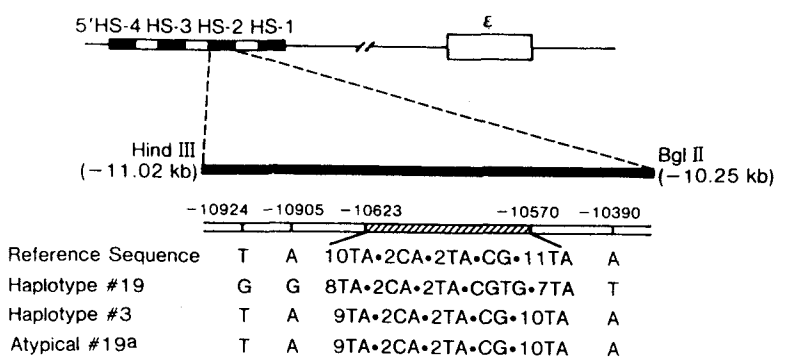


Figure 9. Sequence differences in the LCR HS-2 region of β^S chromosomes with haplotypes Nos. 19 and 3 (from ref. 69).

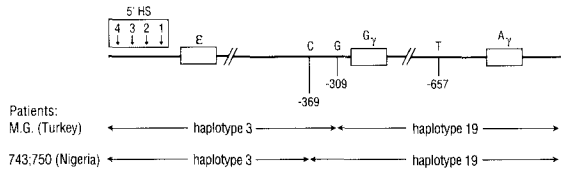


Figure 10. Two different hybrid haplotypes in the Turkish patient M.G. (haplotype No. 19^A) and the two Nigerian patients listed in table 2 (haplotype No. 19^B), each with a different location for the crossover between chromosomes with haplotypes Nos. 3 and 19.

mutation at position −657 5′ to $^A\gamma$, both characteristic of haplotype No. 19, were present. Analysis of the HS-2 of this patient showed that the purine/pyrimidine repetitive sequence was as observed for a β^S chromosome with haplotype No. 3. Thus, the LCR of this atypical haplotype No. 19 chromosome might have arisen by a crossover between positions −309 and −369 5′ to the $^G\gamma$ gene involving chromosomes with haplotypes Nos. 3 and 19 (see fig. 10).

Adekile et al.[2] have found two SS patients from a Nigerian family with a moderate elevation of Hb F (11-19%) and a marked elevation of $^G\gamma$ (>70%); both patients appeared homozygous for haplotype No. 19 mutations and did not have the C→T mutation at −158 (Xmn I negative) (table 5). The mutations in the $^G\gamma$ and $^A\gamma$ promoters listed above (including C→G −369 $^G\gamma$) of both chromosomes in each of the two patients were consistent with haplotype No. 19, while data for the HS-2 suggested heterozygosity for structures specific for haplotypes Nos. 3 and 19. This observation indicated that these patients had one β^S chromosome with haplotype No. 19 and a second chromosome that resulted from a crossover between positions −369 and the ε gene involving chromosomes with haplotypes Nos. 3 and 19 (fig. 10).

The data reviewed above suggest that certain mutations in the HS-2 of the LCR play a significant role in modulating $^G\gamma$ and Hb F expression. According to Öner et al.[69], the unaltered sequence of the HS-2 of the chromosome with haplotype No. 3, as compared to the reference sequence in the GenBank may represent a chromosomal structure that allows for optimal interaction of transacting factors produced under the influence of hematopoietic stress. Conversely, the sequence variations of the HS-2 associated with haplotype No. 19 and/or a combination of yet unknown sequence variations within the LCR may result in a chromosomal structure that is not optimal for γ-globin gene expression during hematopoietic stress.

Mechanism of action of pharmacologic agents stimulating Hb F production

Hydroxyurea, 5-azacytidine, and erythropoietin

Because Hb F inhibits the polymerization of Hb S, there has been a search for pharmacological agents that increase Hb F production in SS patients. Some cytotoxic agents, especially 5-azacytidine and hydroxyurea, were found to have these properties and both have been tried clinically. A third agent that has been tried is the hemopoietic factor, human (recombinant) erythropoietin.

A recent comparative clinical trial[35] of hydroxyurea and erythropoietin indicated that the former increases Hb F production and reduces the rate of hemolysis and intracellular polymerization of Hb S. In contrast, recombinant erythropoietin, whether alone or in combination with hydroxyurea, offered no measurable benefit. Apart from increasing Hb F levels in SS patients, there is evidence that hydroxyurea has significant effects on whole cell deformability and hydration status, leading to an increase in the MCV, a decrease in MCHC, a change toward normality in osmotic ektacytometry, an increase in RBC K^+ content, and a decrease in % dense cells[6,71,73].

The mode of action of hydroxyurea and erythropoietin in increasing the production of Hb F is not quite clear.

Table 5. Hematological data for members of a Turkish family and a Nigerian family with modified β^S haplotypes[a]

Subject	Sex-age	Relationship	Condition	Number of α genes	Hb g/dl	RBC 10^{12}/l	MCH pg	Hb S %	Hb F %	$^G\gamma$ %
Turkish family; homozygous for haplotype No. 19^A										
M.G.	F-8	Propositus	SS	n.d.	12.0	3.85	31.2	-	21.2	64.8
F.G.	M-30	Father	AS	n.d.	17.2	6.19	27.8	34.0	3.3	n.d.
Mo.G.	F-27	Mother	AS	n.d.	14.5	5.38	27.0	37.0	0.7	n.d.
Nigerian family; heterozygous for haplotype No. 19^B										
748	M-52	Father	AS	3	14.1	5.87	24.0	27.2	<2.0	<40.0
749	F-45	Mother	AS	4	11.3	3.88	29.1	39.7	<2.0	61.7
750	F-29	Propositus	SS	3	8.7	3.21	27.1	-	19.0	72.6
744	M-18	Brother	AS	4	14.6	5.18	28.2	37.5	<2.0	76.5
743	F-14	Sister	SS	3	8.8	3.48	25.3	-	10.9	69.7
746	F-7	Sister	AA	3	10.9	4.09	26.7	0	<2.0	35.9

[a]Data are from references 2 and 69; see figure 10 for further information about the two modified haplotypes Nos. 19^A and 19^B.

They both lead to increase in circulating F-reticulocytes and increased numbers of cells per erythroid burst colony-forming unit (BFU-E)-derived colony. Although examination of DNA synthesis in erythroid marrow cells in vitro revealed no decreased methylcytidine incorporation, Eco RI and Hpa II digestion of DNA showed that hypomethylation of γ genes had taken place in vivo after treatment. Charache et al.[18] also showed that 5-azacytidine treatment is associated with non-random hypomethylation of DNA around the γ-δ-β-globin gene complex. Constantoulakis et al.[20] have demonstrated that treatment with erythropoietin, 5-azacytidine, hydroxyurea or butyrate results in induction of γ gene expression as documented by measurement of F-reticulocytes, the $\alpha/(\beta + \gamma)$ biosynthetic ratio, and the level of steady state γ mRNA.

Conclusions

This short review demonstrates that Hb F levels in patients with SS disease differ drastically from those seen in normal individuals who have Hb F percentages below 1%. The increase in the former is quite variable and often dramatic because Hb F levels above 25% can be found in adult patients with a homozygosity for the β^S mutation. There are at least five factors that influence the in vivo expression of the γ-globin genes in these patients. First, the age of the individual; Hb F levels remain far above the percentages observed in normal persons of comparable age, and this elevation is independent of sex, α-globin gene status, and haplotype. Presumably, a severe hematopoietic stress exerts its effect in these young growing SS patients. Second, there appears to be a difference between male and female patients, perhaps because of a yet ill-defined factor that finds its origin on the X chromosome. Third, the coexistence of a severe α chain deficiency decreases the level of Hb F, presumably because the formation of $\alpha\beta^S$ dimers is preferred over that of $\alpha\gamma$ dimers when that α chain pool is greatly decreased. Fourth, mutations within the promoter regions of the $^G\gamma$- and $^A\gamma$-globin genes are directly related to an increased production of $^G\gamma$ chains and an elevated Hb F level. Mutations in the $^G\gamma$ promoter, as seen on β^S chromosomes with haplotypes Nos. 3 and 31 (Senegal and Saudi Arabia-India), appear to be particularly effective. Fifth, most recent data have indicated that sequence differences in controlling regions 5′ to the ε-globin gene (the LCR sequences) are related to elevated Hb F levels as is particularly evident from observations made in a few patients with modified β^S haplotypes, specific LCR sequences, and high Hb F levels.

These data make a search for agents that are able to stimulate γ chain production in vivo most exciting and data from recent studies, including clinical observations, suggest that advances are being made. Such studies should consider the various factors listed and discussed above because these will affect the outcome of clinical trial data collections and will provide the insight needed to explain observations that have been and will be made.

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