

Genetic modulators of sickle cell disease

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ABSTRACT: Sickle cell disease is a monogenic hereditary disease characterized by a mutation in the β -globin gene. The molecular nature is substitution of valine for glutamic acid at the sixth amino acid position in the β -globin gene. The clinical heterogeneity of the disease, which is characterized by the presence of hemoglobin S, can be explained in terms of fetal hemoglobin (HbF) levels, ratio of $\text{G}\gamma$ chains to $\text{A}\gamma$ chains, linked mutations, β^s haplotypes, co-existence of α -thalassemia and environmental factors. Five major haplotypes associated with the β^s mutation have been defined: Benin, Bantu, Senegal, Cameroon and Arab/Indian. The inheritance of Senegal and Arab/Indian haplotypes is associated with a milder course; whereas the Bantu, Cameroon, and Benin haplotypes are linked with severe sickle cell disease. Bantu haplotype carries the worst prognosis of all. These DNA polymorphisms also assume great importance as anthropologic and genetic markers. High levels of hemoglobin F protect against several clinical features. α -thalassemia coincides with sickle cell disease, this gene lowers the mean cell Hb concentration, inhibiting polymerization and hence sickling, giving rise to a milder course. The present review discusses the different genetic modulators that ameliorate the clinical picture of sickle cell disease.

Key Words: Sickle cell disease, β -globin gene, Haplotypes, Genetic modulators.

INTRODUCTION

Sickle cell disease was first noted to affect erythrocytes in 1911, when James Herrick and a medical student of African descent discovered sickle-shaped erythrocytes while examining the student's blood. Linus Pauling further categorized the disorder by noting an abnormal hemoglobin electrophoretic pattern, designated hemoglobin S, and declaring it a molecular disease. The molecular nature is substitution of valine for glutamic acid at the sixth amino acid position at the β -globin chain of hemoglobin. *Hb S* is inherited with an autosomal recessive manner; either two copies of *Hb S* or one copy of *Hb S* plus another β -globin variant (such as *Hb C*) are required for disease expression. *Hb S* carriers are protected from malaria infection, and this protection probably led to the high frequency of *Hb S* in individuals of African and Mediterranean ancestry. Despite this advantage, individuals with sickle cell disease exhibit significant morbidity and mortality. Symptoms include chronic anemia, acute chest syndrome, stroke, splenic and

renal dysfunction, pain crises, and susceptibility to bacterial infections. Pediatric mortality is primarily due to bacterial infection and stroke. In adults, specific causes of mortality are more varied, but individuals with more symptomatic disease may exhibit early mortality. Disease expression is variable and is modified by several factors, the most influential being genotype. Other factors include β -globin cluster haplotypes, α -globin gene number, and fetal hemoglobin expression. In recent years, newborn screening, better medical care, parent education, and penicillin prophylaxis have successfully reduced morbidity and mortality due to *Hb S*.

Nomenclature

Small differences in nomenclature may signify a large difference in patient outcome. The term *sickle cell disorder* encompasses all states in which at least one sickle gene is inherited. This group includes all patients with a positive sickle preparation smear; the patient may or may not be symptomatic. *Sickle*

Table 1. Molecular defects of β -thalassemia in the most common genotypes of sickle cell- β thalassaemia³.

Phenotype	HbA level	Black	Mediterranean	Indian
S β^0 thalassaemia	0	IVS2-849(A→G) FS6(-A) IVS2-1(G→A)	Codon39(C→T) IVS1-1(G→A) FS6(-1bp)	FS14/15(+G) 619bp deletion
S β^+ thalassaemia				
Type I	3-5%	Hb Monroe	IVS2-745(C→G)	IVS1-5(G→C)
Type II	8-14%	-	IVS1-110(G→A)	-
Type III	18-25%	-29(A→G) -88(C→T) PolyA (C→T)	IVS1-6(T→C)	-

cell disease is any type of sickle cell disorder in which significant morbidity, such as organ failure or vaso-occlusive pain crisis (VPC), results from the sickling of red blood cells. The term *sickle cell anemia* is usually reserved specifically for patients homozygous for haemoglobin S (hemoglobin SS)¹. The principal genotypes include:

Homozygous sickle cell disease	SS
Sickle cell-hemoglobin C disease	SC
Sickle cell- β^0 thalassaemia	S β^0 thal.
Sickle cell- β^+ thalassaemia type I	S β^+ thal, Type I
Sickle cell- β^+ thalassaemia type II	S β^+ thal, Type II
Sickle cell- β^+ thalassaemia type III	S β^+ thal, Type III

The subtypes of sickle cell- β^+ thalassaemia represent phenotypes with different expression according to the amount of HbA produced. Type I is a severe defect associated with 3-5% HbA, most commonly seen in Indian patients, type II has higher levels of HbA but remains relatively severe and occurs around the Mediterranean, and type III has 18-25% HbA, runs a mild course and is the type most frequently seen in patients of African origin^{2,3}. (Table 1).

In addition to these major genotypes, three other much less common conditions manifest features of sickle cell disease².

Sickle cell-hemoglobin/D Punjab, Sickle cell-hemoglobin/O Arab, Sickle cell-hemoglobin/Hb Lepore Boston.

Molecular and pathophysiological aspects

Hemoglobin S variant is the product of a single point mutation in which the codon determining the amino

acid at position β^6 has changed from GAG coding for glutamic acid to GTG coding for Valine⁵. A person inherits one β chain gene from each parent in a mendelian autosomal recessive fashion. With a few minor exceptions, people who have only one gene for hemoglobin S are phenotypically normal (sickle trait, HbAS). Deoxygenated Hb S tends to polymerize non-covalently into long strands that deform the erythrocyte, giving the characteristic «sickle cell» morphology (Figure 1)⁶. HbS with bound oxygen (e.g., in the arterial circulation) does not polymerize.

The mechanism by which these changes in the physical properties of haemoglobin S molecule produce the clinical manifestations of the disease is not unequivocally proven. The most widely accepted hypothesis is that erythrocytes deform as they release their oxygen in the capillaries and are trapped in the microcirculation⁷. The blockade of blood flow produces areas of tissue ischemia, leading to the myriad of clinical manifestations seen in sickle cell disease. Recently, investigators have focused on other factors outside the red cell, which could contribute to the manifestations of sickle cell disease. Hebbel and colleagues first showed that sickle erythrocytes adhere abnormally to vascular endothelial cells⁸. Their observations were confirmed and extended by other workers. The endothelial cells may abnormally express adhesion receptors, perhaps in response to activators released from sickle red cells (e.g., reactive oxygen species)⁹. Other investigators have focused on leucocytes and platelets, which might also contribute to disturbed blood flow in sickle cell disease¹⁰. The

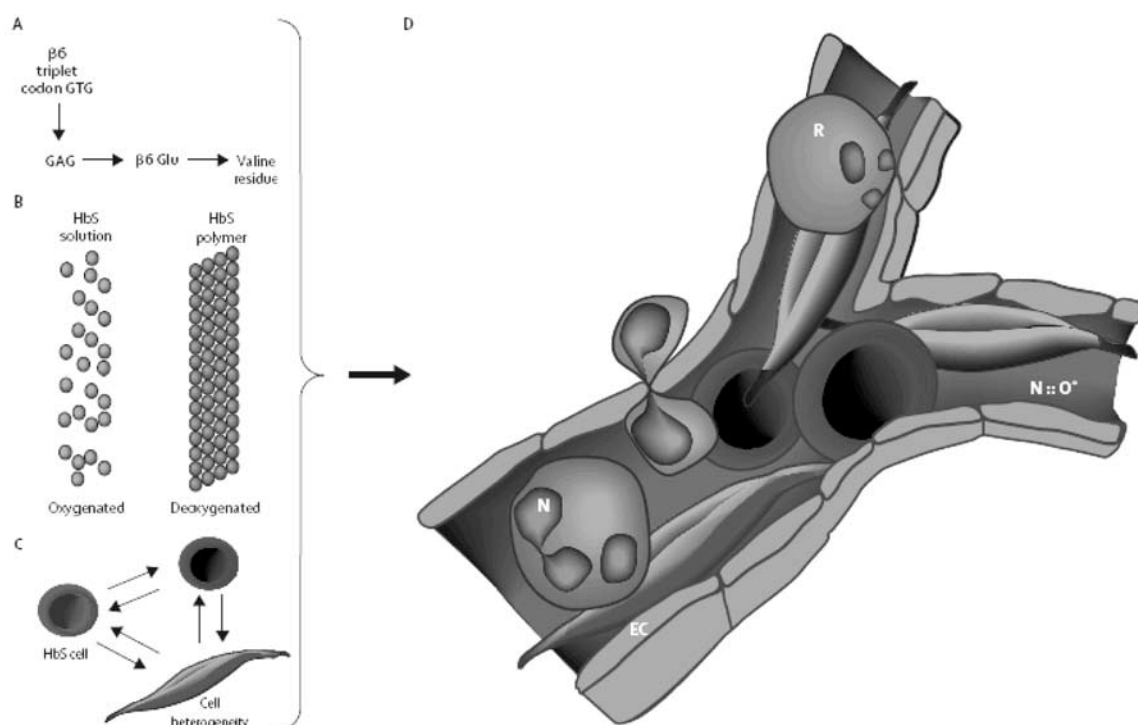


Figure 1. Pathophysiology of vaso-occlusion. (A) Single nucleotide substitution (GTG for GAG). (B) HbS polymerisation. (C) Cell shape changes of HbS-polymer-containing erythrocyte. (D) Cross-section of microvascular bifurcation. EC=endothelium. R=reticulocyte. ISC=irreversibly sickled cell. N=leucocyte. N::O⁺=NO bioavailability. RBC=red blood cell. Luminal obstruction has been initiated by attachment of proadhesive reticulocyte to endothelium with secondary trapping of irreversible sickled cells. Leucocytes participate in formation of heterocellular aggregates, and NO bioavailability crucial to vasodilation is impaired. Figure adapted from reference 7.

involvement of multiple components of the blood in the manifestations of sickle cell disease makes understanding the pathophysiology more difficult. On the other hand, these additional modulators could be targeted by new therapies, with diminution in the severity of sickle cell symptoms.

Gene structure

The haploid genome contains approximately 3×10^9 base pairs and this genetic material is distributed among 23 chromosomes. Of the genes determining hemoglobin structure, the α and the γ genes are duplicated. The α genes code for identical polypeptides, but the γ -chains yield products, which differ in a single amino acid, the globin chain with glycine at position 136 being called $^G\gamma$, and that with alanine $^A\gamma$. On the short arm of chromosome 16 are the ζ - and two α -globin genes and on the short arm of chromosome 11 are the ϵ , both γ ($^G\gamma$ and $^A\gamma$), δ , and β genes (Fig-

ure 2). The entire nucleotide sequence of all human globin genes is now known⁹.

Origin and distribution of the sickle cell gene

Two factors are of prime importance in determining the origin and distribution of the sickle cell gene: the occurrence of the sickle cell mutation and its subsequent selection. The cause of the sickle cell mutation is unknown, but there is evidence that the β^s gene in Africa is presenting three major distinct African haplotypes all localized exclusively to one of three separate geographical areas, i.e. Senegal, Benin and Bantu⁹ (Figure 3).

Recently, a fourth African haplotype, the Cameroon, differing in both the 5' and 3' regions, has been found in the Eton ethnic group. This evidence for these comes from the use of restriction endonuclease enzymes which recognize polymorphic sites (DNA sequence sites that differ among individuals), locat-

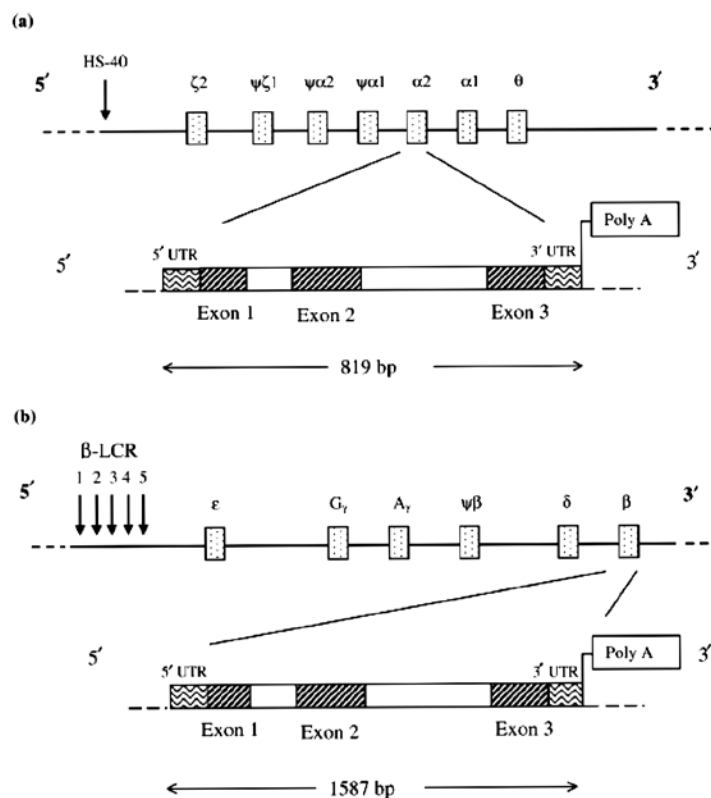


Figure 2. The α - and β -like globin gene clusters, with each globin gene having three exons and two introns. (a) The alpha locus on chromosome 16 p13.3. Of the seven genes shown only three genes are expressed at a clinically significant level, namely the $\zeta 2$ gene (expressed early in foetal development) and the $\alpha 1$ and $\alpha 2$ genes (the focus of most haemoglobinopathy tests). The other genes shown are pseudogenes ($\psi\zeta 1$, $\psi\alpha 1$ and $\psi\alpha 2$) or are not expressed at a significant level (θ). The vertical arrow indicates the location of the upstream hypersensitive site (HS-40), important for locus gene expression. The $\alpha 2$ gene structure is shown below indicating the three coding exons (striped boxes), the two introns (open boxes), and the untranslated regions (zigzag boxes) common to all globin genes. (b) The beta locus on chromosome 11 p15.4 with the ϵ , $G\gamma$ and $A\gamma$, δ and β genes, arranged in the order of their developmental expression. During development two switches are made in beta like globin expression. The first occurs early in foetal development from ϵ globin to the γ globin genes (both $G\gamma$ and $A\gamma$) at 6–8 weeks gestation, and the second switch occurs shortly prior to birth, from the γ genes to the δ and β genes. Again $\psi\beta$ denotes a non expressed pseudogene. Upstream of the beta globin cluster is the β locus control region (β LCR) comprised of the five hypersensitive sites indicated by vertical arrows. As in panel A, the β -globin gene is expanded to show the gene structure covering 1.6 kb. This is bigger than the alpha genes because of a larger second intron.

ed within the β -like gene cluster between the ϵ gene and the β gene and have a very high probability of coinheritance with mutations of β -gene. These sites, when considered as a set, are called *haplotypes*. With a series of enzymes detecting different «recognition» sites it has been possible to define a specific haplotype, characteristic of a population. These ancestral DNA structures are believed to predate the appearance of the sickle cell mutation, and the association of the specific mutation with different haplotypes, although explicable by events such as gene conver-

sion, is most likely due to separate mutational events affecting each haplotype¹¹⁻¹³. (Figure 4).

Studies of DNA from sickle cell populations in Arabia and India revealed the sickle cell gene to be associated with an entirely different haplotype not seen in African populations. This haplotype has been recognized in Arab populations from the Eastern Province of Saudi Arabia¹⁴ and Qatar¹⁵ and from Indian populations in western Orissa State¹⁶. Haplotype analysis in other sickle cell populations has indicated the Benin haplotype to be associated with the β^s gene

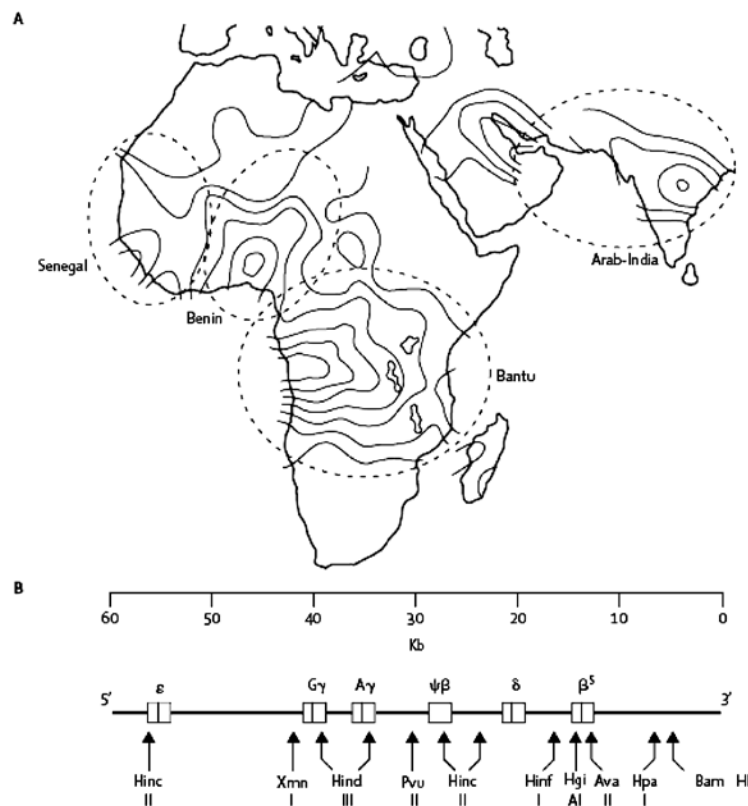


Figure 3. Geographical distribution and schematic representation of the sickle gene. (A) Map identifies the three distinct areas in Africa and one in the Arab-India region where the sickle gene is present (dotted lines). Numbers of individuals with sickle-cell disease (red lines) in Senegal, Benin, and Bantu are higher near the coast, and falls concentrically inland. (B) The β -globin gene cluster haplotype is determined by DNA polymorphic sites (boxes) that are identified by endonuclease enzymes. With this information, haplotypes are constructed as shown.⁰

in Algeria¹², Greece¹⁷, Sicily¹⁸, and Turkey¹⁹ and for the Bantu haplotype to characterize the sickle cell gene in Kenya²⁰. The wide distribution of the Benin haplotype has been attributed to population movement by trans-Saharan migration to North Africa and hence across the Mediterranean, although it is theoretically possible that independent hemoglobin S mutations affected the Benin haplotype structure in different areas. In conclusion there is reasonable evidence that the sickle cell mutation arose independently in at least four locations, three in West and Central Africa and one in Asia (Saudi Arabia and/or India).

Malaria and the sickle cell gene

The high frequency of the hemoglobin S gene in some populations reflects the protection it provides against malaria^{20,21}. The malarial parasite does not survive as

well in the erythrocytes of people with sickle trait as it does in normal people. The basis of the toxicity of sickle hemoglobin for the parasite is unknown. One possibility is that the malarial parasite produces extreme hypoxia in the red cells of people with Hb S trait. These cells then sickle and are cleared (along with the parasites they harbour) by the reticuloendothelial system²². Another possible mechanism is that low levels of hemichromes are formed in sickle trait erythrocytes. Hemichromes are complexes that contain heme moieties that have dissociated from hemoglobin. Hemichromes catalyze the formation of reactive oxygen species, such as the hydroxyl radical, which can injure or even kill the malarial parasites²³.

The malaria hypothesis maintains that during prehistory, on average, people without the sickle gene died of malaria at a high frequency. On the other

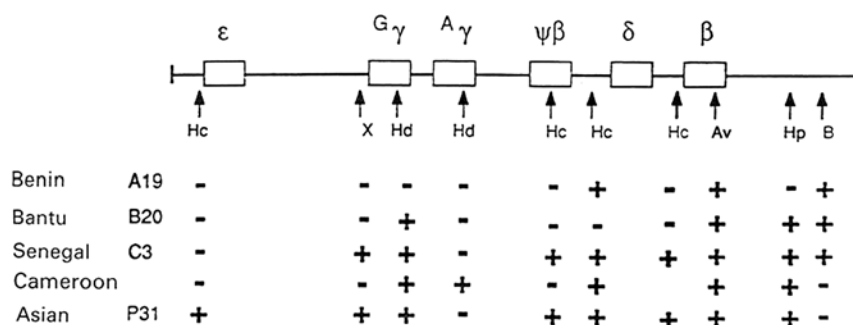


Figure 4. Beta globin haplotypes associated with SS disease in four populations (A19 etc. refer to classification of Antonarakis et al.)¹³.

hand, people with two genes for sickle hemoglobin died of their disease. In contrast, sickle heterozygotes were more resistant to malaria than normals and in addition, suffered none of the ill effects of sickle cell disease. This selection for heterozygotes is termed «balanced polymorphism». Support for this concept comes from epidemiological studies in malaria-endemic regions of Africa. The frequency of sickle cell trait is lower in people coming for treatment to malaria clinics than is seen in the general population²⁴. The reasonable assumption is that relative protection from malaria is at work in this situation.

Genetic modulators of sickle cell disease

Fetal hemoglobin

Augmented post-natal expression of fetal hemoglobin (HbF) is perhaps the most widely recognized modulator of sickle cell disease severity. Fetal hemoglobin, as its name implies is the primary hemoglobin present in the fetus from mid to late gestation²⁵. The protein is composed of two alpha-chains and two gamma-chains. The gamma-chain is a protein product of the β -gene cluster. Duplicate genes, upstream of the β -globin gene encode fetal globin. Fetal hemoglobin binds oxygen more tightly than hemoglobin A. This characteristic allows the developing fetus to extract oxygen from the mother's blood supply²⁶. After birth, this function is no longer necessary and the production of the gamma-chains decreases as the production of the β -globin chains increases. The β -globin chain replaces the gamma-globin chain in the hemoglobin tetramer so that eventually adult hemoglobin (HbA) replaces fetal hemoglobin as the primary component of red cells.

Increased levels of fetal hemoglobin are associated with less severe clinical course of sickle cell disease²⁷. Some individuals have a genetic predisposition to unusually high levels of fetal hemoglobin due to adult overexpression of γ -globin chains. However, this rare condition, hereditary persistence of fetal haemoglobin (HPFH), is only found in one of every 188,000 individuals with sickle cell disease^[28]. Among the majority of individuals with sickle cell disease, who do not have hereditary persistence of fetal hemoglobin, the residual levels of fetal hemoglobin vary considerably. Approximately 40 percent of this variation is accounted for by the X-linked F-cell production locus, and the β^s cluster haplotypes account for an additional 14 percent of the variation²⁹. Some therapies for sickle cell disease, such as hydroxyurea, act for a great part by raising fetal hemoglobin levels in affected individuals. Two properties of fetal hemoglobin help moderate the severity of sickle cell disease. First, HbF molecules do not participate in the polymerization that occurs between molecules of deoxyHbS³⁰. The gamma-chain lacks the valine at the sixth residue that interacts hydrophobically with HbS molecules. HbF has also other sequence differences from HbS that impede polymerization of deoxyHbS. Second, higher concentrations of HbF in a cell allow lower concentrations of HbS. Polymer formation depends exponentially on the concentration of deoxy-HbS.

The level of HbF needed to benefit people with sickle cell disease is a key question to which different studies give varying answers. Bailey *et al.*³¹ examined the correlation between early manifestations of sickle cell disease and fetal hemoglobin level in Jamaicans.

They concluded that moderate to high levels of fetal hemoglobin (5.4-9.7% to 39.8%) reduce the risk for early onset of dactyls, painful crises, acute chest syndrome, and acute splenic sequestration. Platt *et al.*³² examined predictive factors for life expectancy and risk factors for early death among Black Americans and found that, a high level of fetal hemoglobin (>8.6%) secured improved survival. Koshy *et al.*³³ reported that fetal hemoglobin levels above 10% were associated with fewer chronic leg ulcers in American children with sickle cell disease.

Other studies, however, suggest that protection from the ravages of sickle cell disease occurs only with higher levels of HbF. In a comparison of data from Saudi Arabs and Jamaicans and Black Americans, Perrine *et al.*³⁴ found that serious complications (i.e. jaundice, splenectomy, hematuria) occurred only 6% to 25% as frequently in Saudi Arabs as compared to North American Blacks. In addition, mortality under the age of 15 was 10% as great among Saudi Arabs. Further, these patients experienced no leg ulcers, their reticulocyte counts were lower and their hemoglobin levels higher on average. The average fetal hemoglobin level in the Saudi patients ranged between 22-26.8%. This is more than twice the level in the studies mentioned above. Kar *et al.*³⁵ compared patients from Orissa State, India to Jamaican patients with sickle cell disease. These patients also had a more benign course when compared with Jamaican patients. The reported protective level of fetal hemoglobin in this study was on average 16.64%, with a range of 4.6% to 31.5%. Interestingly, β -globin locus haplotype analysis showed that the Saudi HbS gene and that in India have a common origin.

The studies mentioned above suggest that the level of fetal hemoglobin that protects against the complications of sickle cell disease depends strongly on the population group in question. Among North American blacks, fetal hemoglobin levels in the 10% range ameliorate disease severity. The higher average level of fetal hemoglobin could contribute to the generally less severe disease in Indians and Arabs. The investigators have attributed the variability seen to uneven distribution of fetal hemoglobin in erythrocytes. Other observations generally supported this line of reasoning. Patients who were asymptomatic

or virtually asymptomatic had HbF present in most of their erythrocytes. In contrast, patients with markedly uneven distribution of HbF tended to be more symptomatic. The mean level of fetal hemoglobin in the circulation is important. However, the distribution of fetal hemoglobin between the cells is also significant. Heterogeneity of HbF distribution means that some cells will have none of the protective fetal hemoglobin. These cells would be prone to sickling, and could occlude the microcirculation, blocking the flow of cells that normally might have made it across the circulatory net.

Alpha-Thalassemia

Most α -thalassemias results from gene deletion. A normal person inherits two α -globin genes from each parent to give a genotype of $\alpha\alpha/\alpha\alpha$. Deletion of one gene of the pair of closely linked α -globin genes results in α^+ thalassemia, which may be inherited from one ($\alpha^-/\alpha\alpha$) or both (α^-/α^-) parents. Deletion of both α -globin genes results in α^0 thalassemia, which may be inherited from one ($-/\alpha\alpha$) or both ($-/-$) parents. The resulting genetic combinations are summarized in (Table 2). The most common alpha thalassemia in people of West Africa origin is the $-\alpha^{3.7}$ or rightward deletion, which removes 3.7 kb including part of the α_2^- and α_1^- -genes with the intervening DNA. Less common is the $-\alpha^{4.2}$ or leftward deletion, which removes the entire α_2^- -gene. Non deletional α -thalassemia may also occur in which the genes are present but not functioning normally.

Embury *et al.*³⁶ examined the effect of concurrent alpha-thalassemia and sickle cell disease. Based on prior studies, they proposed that alpha-thalassemia reduces intraerythrocyte HbS concentration, with a consequent reduction in polymerization of deoxyHbS and hemolysis. They investigated the effect of alpha gene number on properties of sickle erythrocytes important to the hemolytic and rheological consequences of sickle cell disease. Specifically they looked for correlations between the alpha gene number and irreversibly sickled cells (ISCs), the fraction of red cells with a high hemoglobin concentration (dense cells), and red cells with reduced deformability. The investigators found a direct correlation between the number of alpha-globin genes and each of these indices. A primary effect of alpha-thalassemia was reduction in the

Table 2. Summary of the deletional α -thalassemias³.

Number of globin genes	Genotype	Description	Clinical	Racial group
4	$\alpha\alpha/\alpha\alpha$	Normal	Normal	All races
3	$\alpha\alpha/\alpha-$	Heterozygous α^+ thal	Mild α -thal	Black, Mediterranean, Arab, and South-East
2	$\alpha-/ \alpha-$	Homozygous α^+ thal	»	Asian
2	$\alpha\alpha/--$	Heterozygous α^0 thal	Moderate α -thal	
1	$\alpha/--$	A+thal/ α^0 thal	HbH disease	Mediterranean and
0	$--/--$	Homozygous α^0 thal	Hb Barts hydrops fetalis	South-East Asian

fraction of red cells that attained a high hemoglobin concentration. These dense cells result from potassium loss due to acquired membrane leaks. The overall deformability of dense cells is substantially lower than normal. This property of alpha-thalassemia was confirmed by comparison of red cells in people with or without 2-gene deletion alpha-thalassemia without sickle cell genes. The cells in the nonthalassemic individuals were more dense than those from people with 2-gene deletion alpha-thalassemia. The reduction in median red cell density produced by alpha-thalassemia was much greater in patients sickle cell disease. Reduction in overall hemoglobin concentration due to absent alpha genes is not the only mechanism by which alpha-thalassemia reduces the formation of dense and irreversibly sickled cells. In reviewing the available literature, Embury and Steinburg³⁷ suggested that alpha-thalassemia moderates red cell damage by increasing cell membrane redundancy (morphologically seen as target cells). This protects against sickling-induced stretching of the cell membrane. Potassium leakage and cell dehydration would thereby be minimized.

Ballas *et al.*³⁸ reached different conclusions regarding interaction of alpha thalassemia with sickle cell disease than did Embury *et al.* They too, found that presence of alpha thalassemia reduced the fraction of dense cells but this was associated with increased, not decreased, clinical severity of sickle cell disease. Patients with more highly deformable red cells had more frequent crises. They also found that fewer dense cells and irreversible sickle cells correlated inversely with the severity of painful crises. Inversely, alpha thalassemia was associated with less

severe hemolysis. However, Ballas *et al* reached no clear conclusion concerning alpha gene number and deformability of RBC except to note that the alpha thalassemia was associated with less red cell dehydration.

The two studies are not completely at odds. Both state that concurrent alpha-thalassemia reduces hemolytic anemia. They agree that this occurs through reduction in the number of dense cells, a number directly related to the fraction of irreversibly sickled cells and conclude that through this mechanism red blood cell deformability is increased. The investigators diverge, however, on the relationship to clinical severity of dense cells and rigid cells. Ballas *et al.*³⁸ assert that the reduction of both dense and rigid cells contribute to clinical severity. They advance three possible mechanisms. The most interesting holds that the higher the deformability of cells, the greater their adherence to the endothelium lining the blood vessels. Red cell adhesion to endothelial cells is believed to contribute to vaso-occlusion by retarding erythrocytes in the microcirculation sufficiently long for sickling to occur there^{39,40}. Rigid erythrocytes may or may not enter microvasculature. If they do they are less likely to adhere to the endothelium and cause vaso-occlusion or compromise the blood flow. In contrast, deformable cells have a higher probability of entering the microvasculature, adhering to endothelium and causing vaso-occlusion.

Embury *et al.*³⁶ and Ballas *et al.*³⁸ agree that concurrent alpha-thalassemia and sickle cell disease produce less severe hemolytic anemia through the effect of alpha gene number on HbS concentration, HbS polymer formation, and frequency of dense and irre-

versibly sickled cells. The effect of alpha-thalassemia on other manifestations of sickle cell disease such as painful crises and vaso-occlusion are unresolved.

β-globin cluster haplotypes

There is evidence that the different haplotypes may affect the hematological and clinical expression of sickle cell disease. No differences are currently recognized between the Benin and Bantu haplotypes, although limited information is available. The Senegal haplotype has been claimed to be associated with increased levels of fetal haemoglobin (HbF) although this effect appears to be confined to patients homozygous for the Senegal haplotype. The clinical and genetic significance of the high HbF levels in Senegalese patients with SS disease is difficult to interpret with the data and relatively small number of patients reported¹². The Asian haplotype, on the other hand, appears to be consistently associated with high levels of HbF even in the sickle cell trait^[41]. There are marked hematological implications, with higher hemoglobin levels and lower reticulocyte counts, bilirubin levels, and number of irreversibly sickled cells all consistent with a lower hemolytic rate. The clinical profile is generally a more mild clinical course with fewer vasoocclusive episodes and a greater persistence of splenomegaly.

Most people native to an area indigenous for a particular haplotype are homozygous for that haplotype. In the Americas, mixing among slave populations left most patients with sickle cell disease heterozygous for the two of the three common haplotypes⁴². In a study conducted by Powards *et al.*⁴³, Benin/CAR double heterozygotes trended toward lower fetal hemoglobin levels as well as greater disease severity, while Senegal/CAR haplotypes tended toward intermediate characteristics with respect to fetal hemoglobin level and disease severity. In addition to haplotype, Powards *et al.* examined the effect of gender in patients with sickle cell disease. Females tended to have higher levels of fetal hemoglobin than did males, irrespective of haplotype. The investigators suggested that the higher level of fetal hemoglobin could reflect hormonal factors that interact with a haplotype-specific DNA gene regulatory region. Another possibility is a relative persistence of HbF related to genes located on the X chromosome. Fur-

ther insight into this phenomenon possibly could be gained by examining post-menopausal patients with sickle cell disease in whom hormonal patterns differ from those of younger women.

Although the mechanism by which haplotype is coupled to disease severity is unknown, a correlation clearly exists. Fetal hemoglobin levels vary generally by haplotype and a relation appears to exist between gender, haplotype and HbF levels⁴⁴. This is a relatively new area of investigation with respect to the variability in sickle cell disease and has not been well characterized as alpha-thalassemia and fetal hemoglobin effects. Further investigation could shed additional light on the interplay of haplotypes and disease severity.

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Γενετικοί τροποποιητές της κλινικής έκφρασης της Δρεπανοκυτταρικής Νόσου

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ΠΕΡΙΛΗΨΗ: Η δρεπανοκυτταρική νόσος αποτελεί μία μονογονιδιακή κληρονομική νόσο που χαρακτηρίζεται από μία μετάλλαξη στο γονίδιο που κωδικοποιεί τη β-αλυσίδα της αιμοσφαιρίνης. Η μετάλλαξη οδηγεί σε αντικατάσταση του γλουταμικού οξέος από βαλίνη στη θέση 6 της β-αλυσίδας, με αποτέλεσμα παραγωγή της παθολογικής αιμοσφαιρίνης S. Η κλινική ετερογένεια της νόσου προκαλείται από πολλούς παράγοντες όπως τα επίπεδα της εμβρυϊκής αιμοσφαιρίνης (HbF), το λόγος των ^αγ προς τις ^βγ αλυσίδες, συνδεδεμένες μεταλλάξεις, απλότυποι των β^s αλυσίδων, συνύπαρξη α-μεσογειακής αναιμίας και παράγοντες του περιβάλλοντος. Πέντε μείζονες απλότυποι, συνδεδεμένοι με την μετάλλαξη β^s, έχουν αναγνωρισθεί: Benin, Bantu, Senegal, Cameroon, και Αραβο/Ινδικός (Arab/Indian). Οι απλότυποι Senegal και Αραβο/Ινδικός χαρακτηρίζονται από ήπια διαδρομή. Αντίθετα, οι απλότυποι Bantu, Cameroon και Benin συνδέονται με βαρύτερη κλινική εικόνα. Ο απλότυπος Bantu έχει τη δυσμενέστερη πρόγνωση από όλους τους άλλους. Οι παραπάνω πολυμορφισμοί του DNA προσλαμβάνουν επιπλέον μεγάλη σημασία ως ανθρωπολογικοί και γενετικοί δείκτες. Υψηλά επίπεδα αιμοσφαιρίνης F προφυλάσσουν από τις σοβαρές εκδηλώσεις της νόσου. Η συνύπαρξη α-μεσογειακής αναιμίας προκαλεί ελάττωση της μέσης συγκέντρωσης αιμοσφαιρίνης ανά ερυθρό, με αποτέλεσμα παρεμπόδιση του πολυμερισμού της αιμοσφαιρίνης S και της δρεπάνωσης και ηπιότερη κλινική πορεία. Η παρούσα ανασκόπηση αναφέρεται στους παραπάνω γενετικούς τροποποιητές που βελτιώνουν την κλινική εικόνα της δρεπανοκυτταρικής νόσου.

Λέξεις Κλειδιά: Δρεπανοκυτταρική νόσος, Γονίδιο β-αλυσίδας, Hb απλότυποι, Γενετικοί τροποποιητές.

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