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Review

Variability of homozygous sickle cell disease: The role of alpha and beta globin chain variation and other factors

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ABSTRACT

The single base molecular substitution characterizing sickle cell haemoglobin, β^6glu → val, might be expected to result in predictable haematological and clinical features. However, the disease manifests remarkable diversity believed to reflect the interaction with other genetic and environmental factors. Some of the genetic modifiers include the beta globin haplotypes, alpha thalassaemia, factors influencing the persistence of fetal haemoglobin and the effects of the environment are addressed in this review. It is concluded that much of the genetic data present conflicting results. Environmental factors such as climate and infections, and psychological, educational and social support mechanisms also influence expression of the disease. These interactions illustrate how the expression of a 'single gene' disorder may be influenced by a variety of other genetic and environmental factors.

1. Introduction

Sickle cell disease is a global public health problem affecting over 400,000 births annually [1]. The cause is a single point mutation which changes the behaviour of sickle haemoglobin (HbS) and has been designated as the first molecular disease [2]. The harmless carrier state for HbS known as the sickle cell trait has some protection from falciparum malaria during a critical period in early childhood [3] so that carriers are more likely to survive and pass on their genes. In the presence of falciparum malaria, the sickle cell trait has thus conferred a survival advantage which over the generations, has increased its prevalence to levels as high as 30% in some areas. The prevalence of the trait determines the frequency of sickle cell disease at birth, and the disease is widespread especially in sub-Saharan Africa and central India (Fig. 1). Sickle cell disease is characterized by increased haemolysis and blockage of flow in blood vessels and some of the marked variability in haematological and clinical expression is explained by the different genotypes of the disease resulting from the inheritance of HbS with other interacting haemoglobins. Focusing on homozygous sickle cell (SS) disease which is the single, most common genotype of sickle cell disease at birth, there is widespread diversity in features between different communities and sometimes between different patients in the same family. This report traces the search for the causes of this diversity within a 'single gene' disorder. It concludes that many other factors influence the haematological and clinical expression of the disease and although some of these are genetic, there are also important environmental determinants of severity. Genetic factors and their mode of operation may give valuable insights into new therapeutic approaches but environmental factors may be more readily manipulated. The evidence for these approaches is now presented.

2. Early observations

2.1. The first case reports

The first formal description of sickle cell disease was in a student from Grenada in the West Indies studying dentistry in Chicago between 1904 and 1907 [4]. Nearly 80 years later, the identity of this first patient was learnt from Herrick's original papers as Walter Clement Noel [5]. Although this case report long preceded the development of haemoglobin electrophoresis and more definitive diagnostic procedures, the blood film in Herrick's case leaves little doubt that the patient had homozygous sickle cell (SS) disease. Over the next 12 years, 3 more cases with similar features were reported and Mason [6] reviewed the features of these first four patients noting that all were of African origin (Fig. 2). This led to the common misconception that the disease was confined to persons of African origin and although consistent with the observed distribution of the disease throughout North and South America and the Caribbean, it was later recognized to be common in other racial groups.

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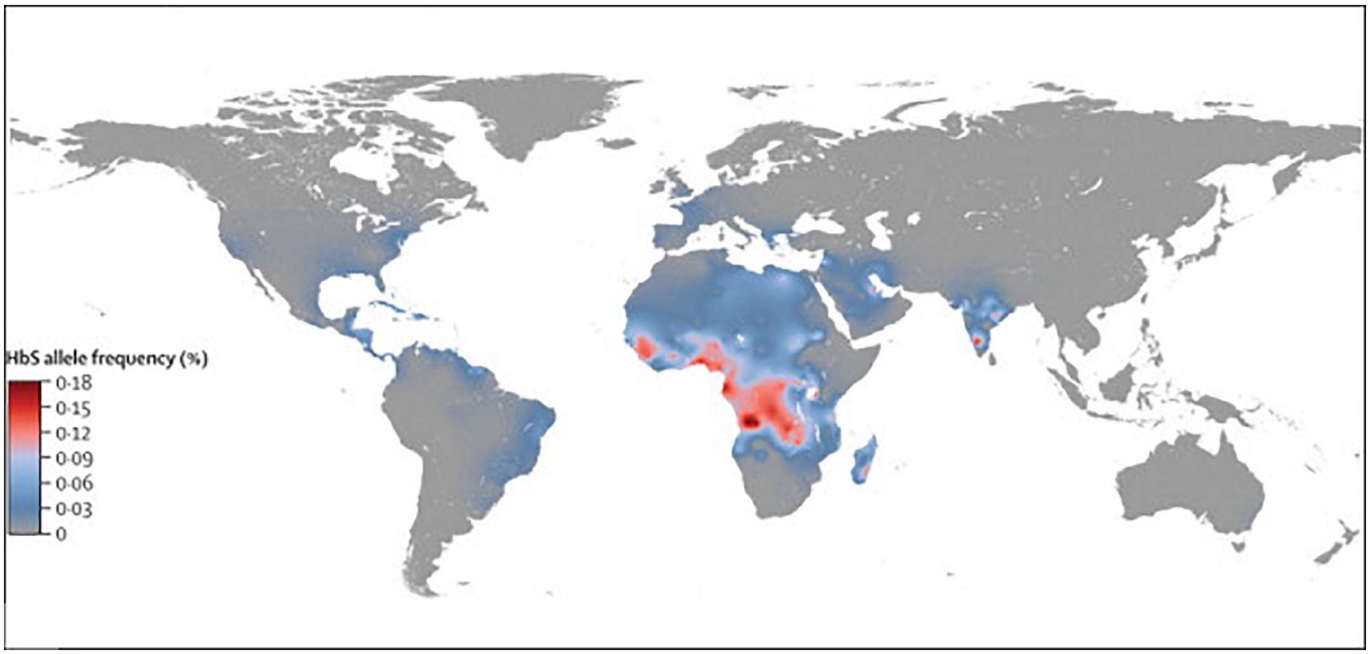
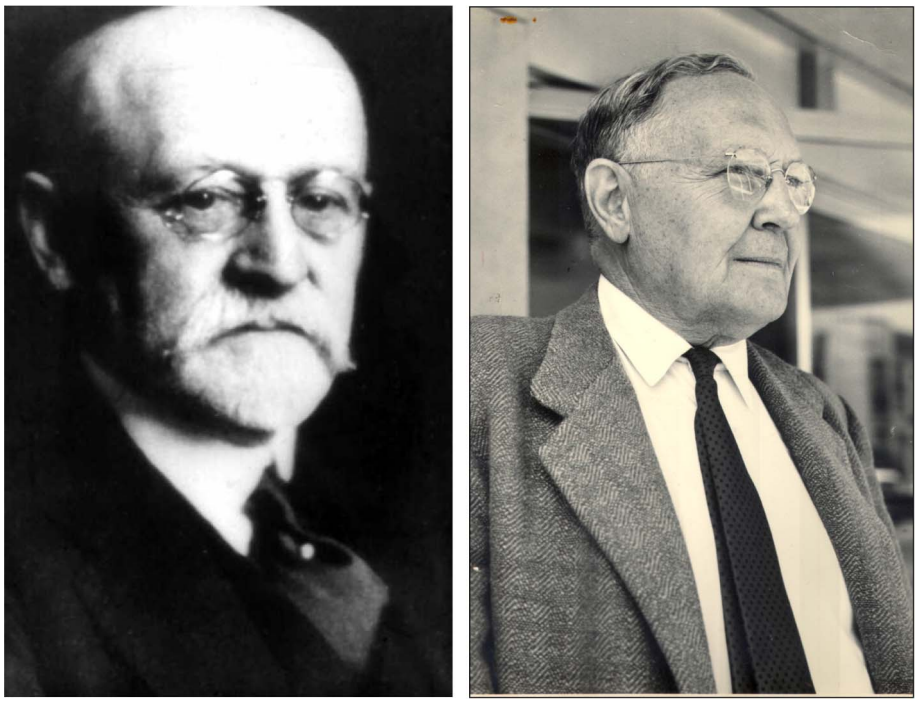


Fig. 1. Map of the distribution of the HbS allele frequency (adapted from the Lancet 2013 and kindly supplied by Dr. Frederic Piel).

2.2. Patterns of inheritance

Confusion emerged from the first studies of inheritance. In the third reported case [7], an early sickle cell test [8] was positive in the patient but also in the asymptomatic father leading to the concept of active and latent sickle cell disease [9]. It was not until 1949 that Neel and Beet working in totally different settings of the United States and the then Southern Rhodesia confirmed that the disease usually resulted from the inheritance of the sickle cell gene from both parents [10,11]. Asymptomatic carriers of the sickle cell gene were termed the sickle cell trait or AS genotype and persons with the disease as homozygotes or SS disease. This inheritance pattern which implied a simple relationship between the frequencies of the sickle cell trait and sickle cell disease in a population did not fit the observations in Africa where high trait frequencies occurred yet few cases of the disease were found [12–14] prompting the former to note that the early death of homozygotes in Africa would represent 'such a slaughter of the innocents that it would not have gone unnoticed'. It soon became clear that such an attrition was indeed occurring [15–17] and homozygous inheritance for much of the disease was confirmed. If both parents have the sickle cell trait, there is a 1 in 4 chance of an offspring with SS disease at each pregnancy (Fig. 3). Regardless of the outcome of each pregnancy, the risks



Dr. James Herrick **Dr. Verne Mason**

Fig. 2. Dr. James Herrick (1861–1954) described the first published case of sickle cell disease in 1910 and Dr. Verne Mason (1889–1965) described the 4th case (see text).

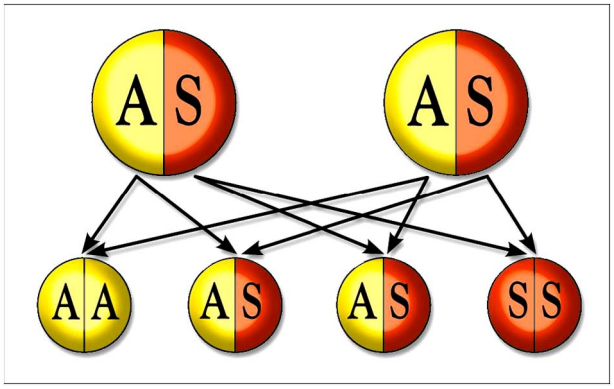


Fig. 3. The expected inheritance pattern where both parents have the sickle cell trait (AS).

remain 1 in 4 and although the chances of the 1 in 4 being followed by the 1 in 4 become increasingly unlikely, sometimes many consecutive births are affected with SS disease (Fig. 4).

2.3. Molecular basis

Elucidation of the change that characterized sickle haemoglobin had to await the development of suitable technologies. Studies on the birefringence of deoxygenated sickled cells [18], the determinants of sickling [19], and the reduction of sickling in young children [20] suggested that it was a feature of adult haemoglobin and Tiselius moving-boundary electrophoresis showed an electrical difference between normal haemoglobin (HbA), the sickle cell trait and sickle cell disease prompting Pauling et al. [2] to suggest that this was a molecular disease. A combination of high-voltage electrophoresis and chromatography indicated an aberrant peptide in HbS [21], chemical analysis showed an excess of valine [22] and sequencing then showed that valine had replaced glutamic acid at position 6 of the beta chain [23] later shown to result from the single nucleotide substitution GAG to GTG [24]. There followed a body of work on the mechanism whereby this substitution led to the pathophysiology of sickle cell disease. This is largely beyond the remit of the present review but there is agreement that deoxygenation of the HbS molecule results in exposure of the mutant amino acid onto the surface of the molecule, promoting polymerization of adjacent molecules, increasing the intracellular viscosity

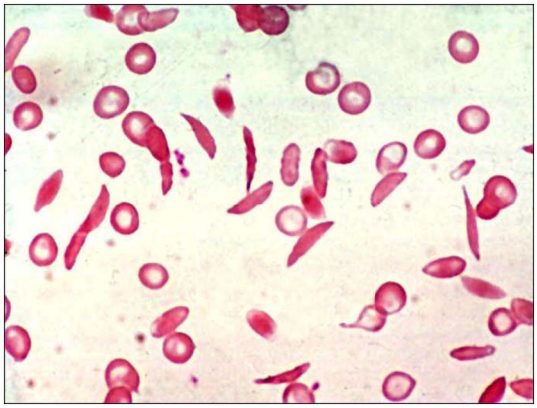


Fig. 5. Peripheral blood film showing the red cell heterogeneity common in patients with SS disease.

and leading to rapid red cell destruction and vaso-occlusion. A feature of the disease is the marked red cell heterogeneity in the peripheral blood, some red cells being dense and irreversibly sickled and others showing variation in size, shape, and haemoglobin concentration (Fig. 5).

2.4. Other variants of sickle cell disease

Although homozygosity for HbS accounted for most of the cases, exceptions continued to appear, some patients with sickle cell disease having a non-sickling parent. Gradually the spectrum of the disease extended to include sickle cell-beta thalassaemia [25], sickle cell-haemoglobin C (SC) disease [26], and the inheritance of the HbS gene along with other interacting haemoglobin variants such as HbD Punjab [27], HbO Arab [28], and Hb Lepore [29].

2.4.1. SC disease

The HbC trait occurs in up to 20% in central Ghana and Burkina Faso in West Africa and falls to 1–2% in surrounding countries; it is not seen in East and Central Africa except in people of West African origin (references summarized by [30]). This focused distribution is usually interpreted as HbC being a recent mutation which has not yet spread far. Because many of the people of African origin in the New World came from this area, the HbC trait occurs in approximately 2% of

Fig. 4. An Indian family in Maharashtra where both parents had the AS genotype but the first 8 children were SS disease. The picture shows from left the father (AS), mother (AS), the next 6 offspring all SS, the last 2 AS and AA. Of the 8 offspring with SS disease, one was not available for the photograph and one had died. (Family investigated courtesy of Professor Sudam Kate, Pune, India).

African Americans [31] and in 3.5% of Jamaicans [32]. The mutation in HbC affects the same amino acid as HbS but glutamic acid is replaced by lysine, rendering it positively charged relative to HbS and therefore moving even more slowly towards the positive pole in conventional alkali electrophoresis. When inherited with HbS, sickle cell-haemoglobin C (SC) disease is generally milder than SS disease with higher haemoglobin levels, less haemolysis and vaso-occlusion and a generally more benign clinical course. One exception to this pattern is the greater prevalence of proliferative sickle retinopathy in SC disease [33] and the mechanism for this is not yet completely understood.

2.4.2. Sickle cell-beta thalassaemia

The diversity of beta thalassaemia genes contributes to a wide variability in the clinical and haematological features of sickle cell-beta thalassaemia syndromes largely determined by the amount of HbA produced. Conditions with no HbA, depicted as sickle cell-beta^{o} thalassaemia, are generally severe, the more common mutations being IVSII-849A > G, IVSII-1G > A, FS6 (− A) and mutations such as Cd 17 AAG → TAG which creates a new stop codon [34]. Clinically sickle cell-beta^{o} thalassaemia is similar to SS disease although, as in SS disease, some cases are unexpectedly benign. Mutations allowing only 3–5% HbA such as the IVSI-5G > C, designated severe sickle cell-beta^{+} thalassaemia, are common in India and generally run a severe course. Mutations resulting in 10% HbA, such as the IVSI-110G > A and IVSI-108T > C around the Mediterranean do not have enough HbA to inhibit sickling when inherited with HbS and may also run a moderately severe course. A benign form of sickle cell-beta^{+} thalassaemia with 20–30% HbA, results from the promoter region mutations − 29A > G, − 88C > T, and the 3′ mutation polyA T > C which occur in peoples of African ancestry. In the 100,000 newborns screened in the Jamaican Cohort Study, the relative prevalences for sickle cell-beta^{o} thalassaemia was 1 in 7700 and for the mild sickle cell-beta^{+} thalassaemia was 1 in 2900 births [32]. The preliminary laboratory differentiation of these conditions was traditionally achieved by haemoglobin electrophoresis (Fig. 6), although high-performance liquid chromatography is rapidly becoming the method of choice.

2.4.3. Interaction with uncommon variants

Of the many haemoglobin variants described, three (HbD Punjab, HbO Arab and Hb Lepore Boston-Washington) are especially important since when inherited with HbS, they may produce clinically significant disease. HbD Punjab is widespread but at low frequency occurring in approximately 1% of Sikhs in the Punjab [35] and in some cases of sickle cell-HbD Punjab, the HbD Punjab gene was inherited from an English ancestor possibly reflecting the long historical military association of the United Kingdom and India. The mutation replaces glutamic acid at β^{121} with glutamine and sickle cell-HbD Punjab runs a generally severe course similar to SS disease. In HbO Arab, the same amino acid is affected with the insertion of lysine in place of glutamic acid and the distribution appears to be widespread but at low frequencies. Inheritance with HbS results in a syndrome similar to SS disease. Hb Lepore Boston-Washington is also widespread but at low frequencies and results from a fusion of delta and beta globin genes manifest as a mild thalassaemia. Inheritance with HbS results in a generally mild but variable condition. Of the 100,000 screened at birth in the Jamaican Cohort Study there were 2 cases of sickle cell-HbO Arab disease, and one each of sickle cell-HbD Punjab and sickle cell-Lepore Boston-Washington [32].

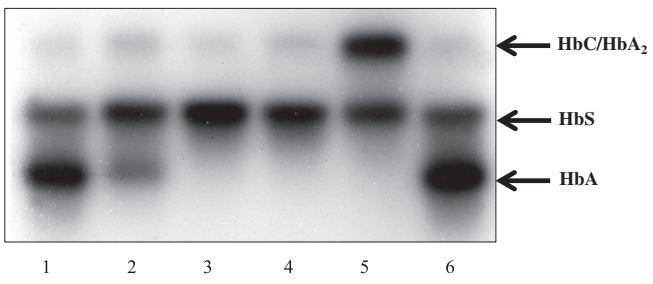


Fig. 6. Alkali haemoglobin electrophoresis in the 4 principal genotypes of sickle cell disease occurring in patients of African origin.
The positive pole is at the lower end of the figure. Lanes 1, 6 sickle cell trait controls, 2 sickle cell-beta + thalassaemia, 3 SS disease, 4 sickle cell-beta^{o} thalassaemia, 5 SC disease.

3. Homozygous sickle cell (SS) disease

3.1. Beta globin haplotypes

3.1.1. African beta globin haplotypes

A further diversity of SS disease appeared with the use of restriction enzymes which identified sequence variations or polymorphisms in the DNA flanking the beta S globin locus (Fig. 7). This work was pursued simultaneously in the United States [36] and in Africa [37]. Antonarakis et al. [36] proposed a numbering system for the different restriction fragment length polymorphisms (RFLP) surrounding the beta globin locus of which the most frequent forms were designated 19, 20, and 3. Work by Dominique Labie at the Institut Pasteur in Dakar, Cotonou, and Bangui, led to these polymorphisms or haplotypes being named as the Senegal (type 3), Benin (type 19) and Bantu (or Central African Republic [CAR]) (type 20) haplotypes of SS disease. These haplotypes were interpreted as independent occurrences of the HbS mutation in Africa [38,39]. The approximate distribution of these haplotypes and their subsequent migration is shown in Fig. 8. A fourth African form known as the Cameroon haplotype (type 17), is limited to the Eton ethnic group of Cameroon [40,41]. Despite a considerable literature in these African beta globin haplotypes, there is still controversy on the extent, if any, they influence expression of SS disease.

3.1.2. Haematological and clinical features in African haplotypes

Comparison of small groups with the Senegal and Benin haplotypes concluded that the Senegal haplotypes had higher levels of HbF, fewer irreversibly sickle cells and higher $^{G}\gamma$ proportions [42–44]. An ameliorating effect of the Senegal haplotype on haematological indices was noted in patients heterozygous for the Senegal haplotype in one study [45] but was only apparent in homozygotes for this haplotype in another [46]. The Bantu haplotype occurred in 91% of 64 SS patients from the Central African Republic and 54 affected patients had HbF levels between those in Senegal and Benin haplotypes, ISC counts were similar to the Senegal haplotype and $^{G}\gamma$% similar to the Benin group [47]. A multi-centre study of 486 patients with SS disease confirmed the distribution of these haplotypes [48] but there was concern on the validity of the haematological indices.

If confusion surrounds the data on HbF levels, $^{G}\gamma$% and total haemoglobin levels, there is even greater confusion on any clinical effects. In southern California, comparison of 28 subjects heterozygous for Senegal/Benin haplotypes, 55 subjects heterozygous for CAR/Benin and 84 subjects homozygous for the Benin haplotype produced some evidence that the Senegal haplotype had less frequent hospital admissions, less ‘sickle cell crisis associated with illness’, and less ‘bone infarcts’ [49,50]. On this basis, they ranked CAR, Benin and Senegal in decreasing order of clinical severity and also suggested that the CAR haplotype was more prone to early death [51] and end-organ damage [52,53]. On the other hand, there were no consistent difference in haematology or clinical features between 53 Benin heterozygotes, 32 CAR heterozygotes and 15 Senegal heterozygotes in the US [54] although the retrospective nature of this study and other concerns may have compromised these findings [55]. However, a French group reporting 37 homozygotes for the CAR haplotype, 57 homozygotes for the Benin and 26 homozygotes for Senegal also found no significant clinical differences [56]. The marked preponderance of the Benin haplotype in the US and Jamaica has rendered these population relatively insensitive

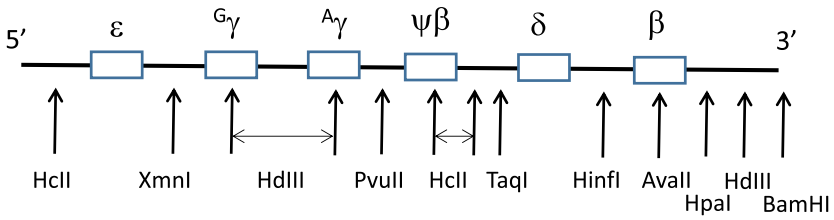


Fig. 7. Depiction of polymorphic sites on chromosome 11, restriction enzymes and resulting beta globin haplotypes.

Senegal	-	+	+	-	+	+	+	+	+	+	+	+	+	
Benin	-	-	-	-	+	-	-	+	-	+	-	-	+	
CAR	-	-	+	-	+	-	-	-	-	+	+	+	+	
Cameroon	-	-	+	+		-	+			+	+		+	
Asian	+	+	+	-	+	+	+	+	-	+	+	+	-	

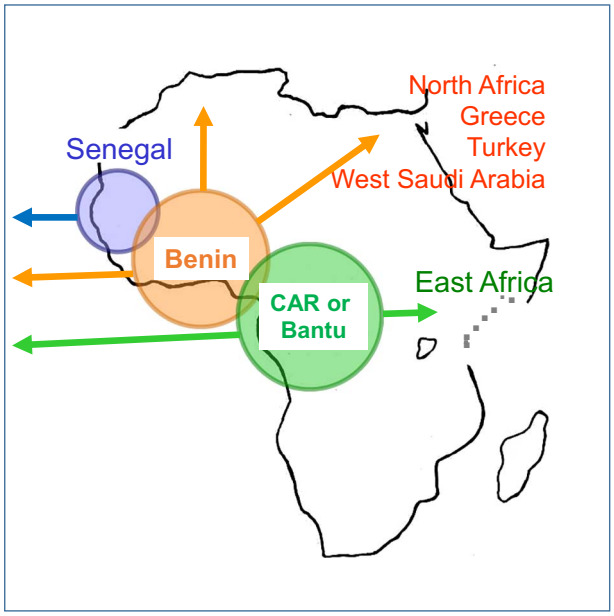


Fig. 8. The African Continent showing the distribution of the 3 major African haplotypes of sickle cell disease.

for assessing the effect of beta globin haplotype. The French data based on homozygotes for the 3 more common haplotypes are probably the most reliable.

3.1.3. *The Asian haplotype (also called the Arab-Indian haplotype)*

A further mutation with a specific RFLP pattern including a restriction site for *Xmn* I was found in the Eastern Province of Saudi Arabia and throughout central and southern India. First described in Jamaica in an extended family of 10 SS subjects with high HbF levels and partial Indian ancestry [57], this haplotype was subsequently recognized to be widespread in India and Eastern Saudi Arabia. In Burla Medical College in western Odisha State, India, a study of 131 patients with SS disease [58] found this haplotype in 124/126 (107 homozygotes) [59]. It also accounted for 91% of the SS patients among the tribal populations of the Nilgiris in southern India [60], in 92% of 70 SS subjects in Gujarat and Maharashtra [61] and in 87% (65% homozygotes) among 100 SS subjects in Chhattisgarh [62]. Overall it is estimated that the Asian haplotype occurs in 92–95% of SS disease in India (Roshan Colah, personal communication 2017). This haplotype is also widespread around the Gulf although within Saudi Arabia it is confined to the Eastern Province (Fig. 9) whereas the South-Western Province has predominantly the Benin haplotype [63–65]. The Asian haplotype has also been described from Qatar [66], Kuwait [67] and in 23% SS subjects from northern Iran [68].

3.1.4. *Haematological and clinical features in the Asian haplotype*

The common association of the Asian haplotype with high levels of HbF and frequent alpha thalassaemia complicates assessment of any role of the Asian haplotype itself. Contrary to the findings of El-Hazmi [63], most studies have found an elevated HbF level in the Asian haplotype. Comparing 32 SS subjects in the Eastern Province of Saudi Arabia with the Asian haplotype (31 homozygotes) with 28 SS subjects from the Southwest (21 had the Benin haplotype, homozygous in 19), the Eastern group had higher HbF and Hb, and lower MCV, all differences being highly significant [65]. However, HbF levels in SS adults homozygous for the Asian haplotype ranged from 4.9–20.4% in 16 Eastern Province Saudi males and 6.0–19.5 in 16 females (GR Serjeant – unpublished data) indicating that other factors influence HbF level. HbF levels in the AS parents of Indian SS patients correlated to the HbF level in their SS offspring [59] and studies in the Eastern Province found that Saudi AS parents generated higher HbF levels in BFU-E's which correlated with HbF levels in their SS offspring [69]. They concluded that these families possessed a genetic factor allowing persistence of HbF synthesis in the presence of accelerated erythropoiesis. Part of the problem in interpreting these data is the imprecision of working with haemolysate HbF levels, which are influenced by red cell selection and a clearer pattern may emerge with more sensitive indicators such as HbF containing cells and HbF reticulocyte counts [70]. This issue will recur later with the genetics of HbF levels.

Clinically, the disease associated with the Asian haplotype is generally more mild [71–73] but although the persistence of splenic function may protect against overwhelming septicaemia, it may render patients more prone to chronic hypersplenism [74]. Padmos et al. [65] also found less dactylitis, jaundice and acute chest syndrome in Eastern compared to South-Western Saudi patients. In Akola, Maharashtra State, India, a study of 49 patients with SS disease showed moderately severe features [75], despite all having the Asian haplotype (46 homozygotes). In several studies of the Asian haplotype, despite some evidence of clinical amelioration, bone pain crises continued to be a major clinical feature although this may be telling us more about the pathogenesis of bone pain.

3.2. *Alpha thalassaemia*

A further source of diversity in the expression of SS disease relates to the number of alpha globin genes. Most people have 4 alpha globin genes, a pair of two closely linked alpha globin genes on each chromosome 16 depicted as αα/αα. A variety of gene deletions may occur, some of which have specific geographic distributions. Deletion of one of the pair of linked genes may be inherited from one parent α −/αα, known as heterozygous α^+ thalassaemia (or α thalassaemia 2) or from

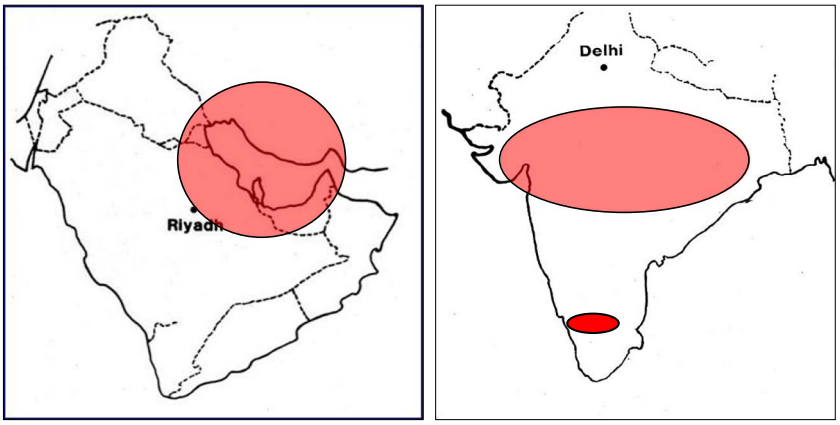


Fig. 9. Distribution of the Asian Haplotype of sickle cell disease.

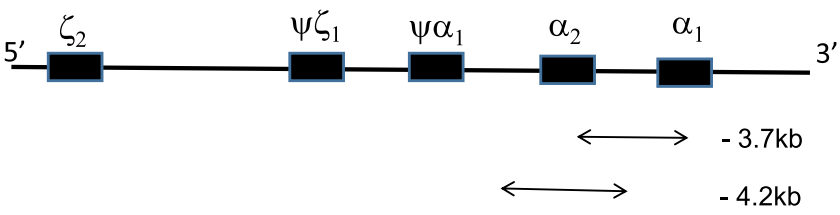


Fig. 10. The a-globin like cluster on chromosome 16 showing the 'rightward' deletion of −3.7 kb and the 'leftward deletion of −4.2 kb which cause common forms of alpha thalassaemia.

both parents α−/α−, known as homozygous α^+ thalassaemia. These forms are commonly found in populations with SS disease and lower the HbS concentration probably inhibiting the polymerization of HbS and hence sickling. Another form of deletion removes both of the pair of linked alpha globin genes and may be inherited from one parent −−/αα, known as heterozygous α^o thalassaemia (or α thalassaemia 1) or from both parents −−/−−, known as homozygous α^o thalassaemia. This gene is very rare among people of African ancestry but occurs in Southeast Asia; deletion of all alpha globin genes is usually not compatible with life resulting in Hb Barts hydrops fetalis. Occasionally the α^+ thalassaemia gene may be inherited with the α^o thalassaemia gene causing α−/−− or HbH disease which has rarely been described in association with HbS. Two forms of deletion may cause common alpha thalassaemia, the 'rightward' or −3.7 kb removes part of both alpha globin genes and is the usual form in patients of African origin so coinciding with SS disease (Fig. 10). The 'leftward' or −4.2 kb occurs in Asia but may be seen occasionally in people of African origin [76]. Non-deletional forms of alpha thalassaemia may rarely be inherited with HbS.

3.2.1. Distribution and frequency

The prevalence of these genes is critically dependent upon sample selection but in the Jamaican Cohort of 100,000 consecutive deliveries, alpha globin gene number was available in 205/246 subjects with a normal AA haemoglobin genotype and indicated 136 (66%) with αα/αα, 62 (30%) with α−/αα, and 5 (2.4%) with α−/α−. Corresponding figures in 268 subjects with an SS genotype were 169 (63%), 90 (34%) and 9 (3.4%) [32]. In African countries, the prevalence of alpha thalassaemia (heterozygotes and homozygotes) among SS patients has varied between 37% and 77% in Uganda [77,78], Cameroon [79], and Tanzania [80] almost certainly reflecting the degree of symptomatic selection in sampled subjects. Frequencies over 50% are usually quoted for combined heterozygous and homozygous alpha thalassaemia in Saudi Arabia [65] and in India [58,81] but levels have been reported as high as 91% in tribal populations from Valsad [82] and as low as 32% [82] and 16% in severely affected patients in Nagpur [75].

3.2.2. Haematological and clinical features of SS disease with alpha thalassaemia

Since alpha thalassaemia lowers the intracellular concentration of HbS which is an important determinant of sickling, it might be expected to reduce haemolysis and protect against vaso-occlusion. However homozygous alpha thalassaemia is also associated with an increased haemoglobin level and the greater viscosity may compromise blood flow in large vessels. The alpha thalassaemia gene might therefore provide a mechanism for distinguishing impairment of capillary flow from that in larger vessels. A study of 47 patients with SS disease (25 αα/αα, 18 α−/αα, 4 α−/α−) found alpha thalassaemia to be associated with higher total haemoglobin and HbF, lower reticulocytes, and lower MCV, MCH, and MCHC [83]. A controlled study of 176 age-matched subjects in Jamaica (88 αα/αα, 44 α−/αα, 44 α−/α−) found that subjects with alpha thalassaemia had higher total haemoglobin, HbA_2, lower HbF, reticulocytes, MCV, MCH. MCHC, irreversibly sickled cell counts, and lower serum bilirubin. Homozygotes with alpha thalassaemia also had less acute chest syndrome and leg ulceration and greater persistence of splenomegaly [84]. With the exception of HbF levels, these two studies and others since have reached similar haematological conclusions, the interaction of alpha thalassaemia with SS disease reducing the haemolytic rate [85], increasing red cell deformability [86] and improving the rheology of HbS containing cells [87] and reducing the number of dense RBC [88].

If there is general agreement on the haematological features of alpha thalassaemia in SS disease, data on the clinical outcome is more controversial. Studies of stroke suggest a protective effect of alpha thalassaemia in some [89,90] but not in others [91,92]. Even with the more extensive data available from the Cooperative Study, there was an overall protective effect but this failed to reach significance when analyzed separately for infarctive and haemorrhagic causes [93]. Cross-sectional studies have shown that alpha thalassaemia may delay clinical presentation [94,95] and an increasing frequency of the alpha thalassaemia gene with advancing age was consistent with improved survival [88,96,97] although no difference was found between neonates and adults from a large study in Guadeloupe [98]. Alpha thalassaemia appeared to increase the risk of avascular necrosis of the femoral or humeral head in a study of 52 adults in the US [99]. Nowhere is this confusion better illustrated than in the possible effect of alpha thalassaemia on the prevalence of the bone pain crisis. Early studies

comparing 176 patients with SS disease in Jamaica [84] or 125 subjects with known alpha globin gene number (13 α −/α −, 39 α −/αα, 73 αα/αα) in the US found no difference in the proportion affected or admitted with bone pain crisis [100]. Alpha thalassaemia reduced dense red cells in 25 SS patients (13 α −/αα, 12 αα/αα) but was associated with increased admissions for painful crisis in the US [101,102]. The Cooperative Study in the US compared 100 SS patients with alpha thalassaemia (either heterozygotes or homozygotes) with 210 patients with 4 or 5 alpha globin genes finding painful crises to be increased in those with alpha thalassaemia [90] similar to Jamaican findings in 637 SS patients (48 α −/α −, 276 α −/αα, 363 αα/αα) [103]. Comparing 75 SS children with ≥ 3 severe pain episodes in the preceding year with 232 without painful episodes found a significant excess of α-thalassaemia trait among the pain group [104] but a French study of 105 SS patients showed no clear reduction of bone pain crises in those with α-thalassaemia [105]. These apparently conflicting effects of alpha thalassaemia was also noted in a review [106].

The lack of stronger clinical associations with alpha thalassaemia may be interpreted as a true lack of effects or more likely the lack of data collected specifically to address this question, controlling for probable other interacting factors and the definition of clear clinical endpoints. Part of the problem with the plentiful data is that much of it has been collected without protocols designed to address the specific question of the influence of alpha thalassaemia on the clinical features of SS disease. In the perfect world, such studies would be designed to control for the many confounding factors and this is well illustrated by the painful crisis which is known to be influenced by age, gender, haemoglobin level, precipitating environmental factors, psychological factors and stress. Controlling for all of these would be virtually impossible but attempts to control for some might produce a clearer picture. Furthermore, the clinical definition and end-point for bone pain (frequency, severity, admission rate, etc.) varies between studies and confusion may also arise from unjustified pooling of groups such as the combining of heterozygous and homozygous alpha thalassaemia as there is some evidence that bone pain may be reduced in heterozygous alpha thalassaemia but increased in homozygotes.

3.3. Fetal haemoglobin

The dominant haemoglobin at the time of birth is fetal haemoglobin (HbF) composed of 2 alpha and 2 gamma globin chains ($\alpha_2\gamma_2$). In normal development, the synthesis of γ chains is progressively replaced by β chains (Fig. 11) resulting in the production of HbA ($\alpha_2\beta_2$) and HbF levels fall to < 1% by 2 years of age [107]. In SS disease, the decline of HbF allows the abnormal β chains bearing the HbS substitution ($\alpha_2\beta^S_2$) to increase to levels causing clinical symptoms around 3 months of age [108]. In SS adults, mean HbF in females was 6.05% and in males 4.93% [109] although levels may rise at later ages in cross-sectional studies [110] probably reflecting the greater survival of patients with high HbF levels. Production of γ chain synthesis is determined by two nearly identical genes in the β-globin gene complex on chromosome 11 (Fig. 7) which differ by the amino acid at γ^{136}, glycine in the $^{G}\gamma$ gene and alanine in the $^{A}\gamma$ gene and their relative ratios have been used as indirect indications of the activity of the two γ genes. In SS disease, HbF levels persist for longer and several genetic factors are known to inhibit the switching-off of γ chain synthesis. The beneficial effects of high HbF levels imply that the mechanism of this switch may be very important.

3.3.1. Factors affecting the switch from γ chain synthesis

Recent quantitative trait loci and genome-wide association studies have identified three major loci influencing HbF including the 158Xmn1, the MYB region of chromosome 6Q23, and BCL11A of chromosome 2p15 [111–113]. In sickle cell disease the transcription factor BCL11A has been most studied. It interacts with many DNA binding proteins to suppress gamma globin expression through binding to the HbF silencing region [114]. A complex genetic network with transcription factors including KLF1, BCL11A, MYB and others regulate this switch [115–125]. Chromosomal looping from the locus control region to the promoters of globin genes repress or activate fetal haemoglobin production [117,126]. Any increase in HbF has a beneficial effect on mortality which appears to be more protective against vaso-occlusive complications than haemolytic associated adverse events [127].

3.3.2. Hereditary persistence of fetal haemoglobin

In the classic pancellular sickle cell-hereditary persistence of fetal haemoglobin (HPFH), an HPFH gene is inherited from one parent and HbS from the other, HbF levels of 15–30% are evenly distributed through all red cells, sickling is inhibited and there is a generally benign clinical course. At the molecular level, persistence of HbF may be associated with single point nucleotide deletions in the promoter region such as the $^{A}\gamma$ −198 T > C or the $^{G}\gamma$ −202 C > G [128] or large deletions > 80 kb remove the delta and beta globin genes. Most common among peoples of African origin, and therefore more likely to be inherited along with HbS, are the HPFH-1 (African form) and the HPFH-2 (Ghanaian form). These have been estimated to occur in 1 in 5000 African Americans but may be more common in Jamaica where among 16,612 school students, HbF levels exceeded 9% in 55 subjects aged ≥ 13 years, and in the 53 subjects sequenced, there were 3 promoter point mutations, 15 with the HPFH-1 trait and 35 with the HPFH-2 trait (GRSerjeant, unpublished data). The complexity of genetic factors causing persistence of HbF has been reviewed [129].

In the heterocellular hereditary persistence of HbF, both parents have the HbS gene but one or both parents also have a modest increase in HbF level which in their SS offspring may result in markedly elevated HbF and sometimes a more mild clinical course. The genetics of these syndromes are complex and not well understood but high levels of HbF from this mechanism characterize some populations especially those

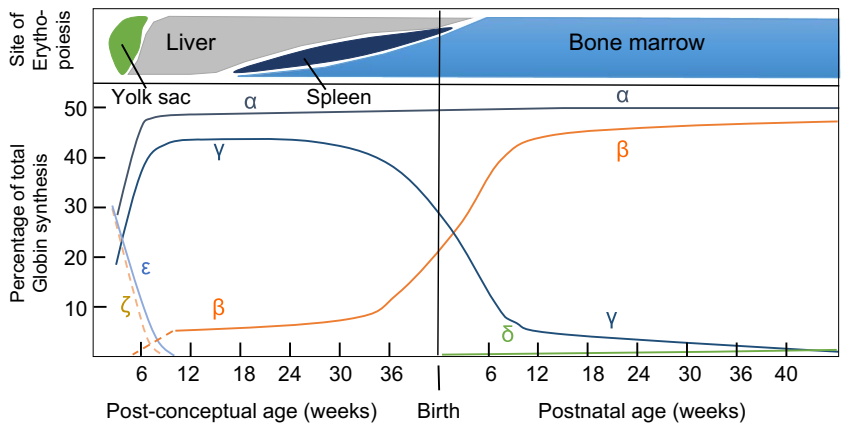


Fig. 11. Site and type of globin chain synthesis in prenatal and postnatal periods.
(adapted from Weatherall & Clegg 1981).

associated with the Asian and homozygotes with the Senegal haplotypes.

3.3.3. Haematological effects of persisting HbF levels

The uneven distribution of HbF levels between red cells complicates interpretation of haemolysate HbF levels which are subject to red cell selection. This is most clearly demonstrated in the aplastic crisis when the production of new red cells temporarily ceases and the greater survival of high HbF containing cells raises the haemolysate HbF level progressively until recovery is signaled by an outpouring of immature red cells with lower HbF levels [130]. Interpretation of HbF levels may also be confounded by patient selection since some patients with high HbF levels run a milder clinical course and so will be increasingly represented in older age groups [110]. In general, high HbF levels correlate positively with total haemoglobin and negatively with MCV and irreversibly sickled cell (ISC counts) but there was no consistent relationship with percentage reticulocyte count [109,131].

3.3.4. Clinical effects of persisting HbF levels

In the Jamaican Cohort Study, low HbF levels at 6 months of age correlated with a history of dactylitis, early splenomegaly, acute splenic sequestration and death [132]. Survival curve analysis in the same study but with more extensive follow-up showed that the protective effects of HbF were confined to males although higher HbF appeared to protect against acute chest syndrome in both sexes [133]. The observation that high HbF levels allow persistence of splenomegaly in SS disease [134] has since been amply confirmed in India [58] and Saudi Arabia [65]. It is still unknown whether persistence of splenomegaly also implies continuing normal splenic function although data from pitted red cells in Saudi Arabia [135] and the rarity or absence of overwhelming blood infection with *Streptococcus pneumonia* (pneumococcal septicaemia) in such populations ([136–138]) is consistent with this. The apparent infrequency of pneumococcal septicaemia in Indian SS patients raises a vital question of whether persistence of splenic function extends beyond the typical age-specificity of pneumococcal septicaemia [139] and offers effective prevention or whether cases are being missed because of mortality prior to investigation or the random use of antibiotics. Since the costs and logistics of effective pneumococcal prophylaxis are considerable, it is vital that this question is addressed urgently.

Persistence of splenomegaly may also be related to chronic hypersplenism although the relationship is still unclear. Hypersplenism occurred in approximately 5% of the Jamaican Cohort but there was no relationship with HbF levels in either sex [133]. Hypersplenism also occurs in India [58] but its prevalence and natural history is unknown. Caution is required in the interpretation of HbF levels in hypersplenism since unless steady state values prior to hypersplenism are available, the extremely short red cell survival imposes marked red cell selection and an elevation of haemolysate HbF levels. Acute splenic sequestration (ASS), although common in the Jamaican Cohort, the relationship with low HbF levels was confined to males [133]. In areas where SS disease is associated with the Asian haplotype and therefore usually higher HbF levels, ASS is known to occur [136,140] but little is known on its prevalence or natural history.

The spectrum of bone marrow necrosis (dactylitis, bone pain crisis, avascular necrosis of the femoral head) and its relationship with HbF levels again lacks clarity. Dactylitis, which is free of the factors that may confound bone pain crisis, occurred in 45% of the Jamaican Cohort by 2 years of age [141] and in the Cooperative Study in the US, was reported in 25% by the age of 2.5 years [90]. Gender prevalence did not differ in either study but the relationship with low HbF level in Jamaica was confined to males [133]. Data in the bone pain crisis are much more difficult to interpret because bone pain is influenced by many factors including age, gender, haematology, environmental, stress, psychological and other factors. Bone pains become frequent in childhood, increasing in prevalence in adolescence especially in males, and generally ameliorating after the age of 30 years [142–144]. Bone pain continues to be a major feature of SS disease even in areas where high levels of HbF reduces the frequency of other pathologies [58,65]. This observation is open to many interpretations, one of which is that the bone pain crisis is not directly related to intravascular sickling [101,103], and that the bilateral, symmetrical distribution of bone pain, and its precipitation by skin cooling may be more readily explained by a centrally mediated reflex shunting of blood away from the bone marrow reminiscent of a 'steal' syndrome [145,146]. Of the other major pathologies of sickle cell disease, the lack of adequate information on prevalence and natural history limit the conclusions which may be drawn but acute chest syndrome may be less likely in areas where high HbF is common [65], stroke certainly occurs but the relative prevalence is unclear, and chronic leg ulceration and priapism are certainly less frequent in areas with the Asian haplotype and elevated HbF levels [65,140]. These data appear to support the concept that higher levels of HbF are generally associated with a milder clinical course and a threshold protective level has been proposed [147]. However, the corollary that low HbF levels are associated with uniformly severe courses was contested by a comparison of haematology and clinical course in groups approximately matched for age and alpha globin gene number of 50 Jamaican patients with HbF levels below 1%, 54 with levels between 2.5 and 3.4% and 60 with levels between 4.6 and 5.2% which concluded that the low HbF group had lower total haemoglobin and mean cell volume but no significant clinical differences between the groups [148].

3.4. Other genetic factors

Genetic studies now indicate a complex interaction of independent genetic modifiers that alter the downstream effects of the sickle cell mutation and polymerization. Historically, sickle cell disease was characterized as a haemolytic anaemia associated with blockages of blood flow but now, it is recognized as a complex inflammatory disease with a systemic vasculopathy. Genetic modifiers that alter inflammatory cell adhesion, coagulation, and vasoregulation significantly affect the physiology of the disease. Independent genetic factors that alter nitric oxide metabolism, iron trafficking, adenosine receptor genes also influence the phenotype expression of the disease. Bilirubin levels and the development of gallstones may be influenced by variants of the UGT1A1 gene. Furthermore, specific end organ complications such as stroke, kidney disease, pulmonary hypertension, cardiovascular complications, and pain sensitivity are all influenced by genetic modifiers.

3.4.1. Developing approaches to genetic modifiers in sickle cell disease

Recent attempts to understand the severity of the sickle cell phenotype has undertaken innovative multiomic analysis with integrated interactions [149]. Transcriptomic studies demonstrating up or down regulation of 400 differentially expressed genes were associated with disease severity [150]. New studies focusing on posttranscriptional mechanisms of gene regulation via micro RNA-mediated processes are uncovering new insights into clinical severity [151]. Proteomic and metabolomic studies in sickle cell disease are revealing important metabolic derangements that may modulate the clinical phenotype [152–156]. Recently, red blood cell interactome analysis and other new methods are being utilized to understand sickle cell dependent changes [154,157–160]. The development of techniques that combine biomarkers from proteomics, transcriptomics, and metabolomics will in the future allow for validated functionally linked biomarkers that predict disease [149].

4. Multi-factorial influences in sickle cell disease

It is clear from the foregoing review that despite the accumulating data from extensive studies, there are few clear messages from the interaction of beta globin haplotypes, alpha thalassaemia, and persistence

of fetal haemoglobin on the expression of the 'single gene' disorder of homozygous sickle cell disease. These conflicting data are difficult to interpret and illustrate the complexity of the relationship between haematological indices and the imprecision of defining the clinical course. In the latter, some messages emerge on the acute chest syndrome, stroke, infections, chronic leg ulceration, priapism and perhaps the cumulative end-organ damage affecting the lungs and kidneys. However, one of the major manifestations of sickle cell disease, the bone pain crisis, remains an enigma with few clear genetically controlled risk factors except perhaps high total haemoglobin and low HbF levels. For the bone pain crisis, it is also clear that environmental factors such as skin cooling may be major determinants in their precipitation, and social and psychological factors influence the patients' ability to cope with pain. The relative roles of genetic and environmental factors in the expression of sickle cell disease was explored in a study of 6 pairs of identical twins which concluded that genetic factors determined haematology and growth but that the clinical course was frequently discordant between the twin pairs [161]. This further strengthens the role of non-genetic influences in expression of sickle cell disease and although this may be scientifically unexciting, identification and avoidance of adverse environmental effects [162,163], such as avoiding cold baths at cold times of day, may have more immediate benefits for the patient.

5. Conclusion

This review focused on a genetic condition resulting from a single nucleotide substitution which might have been expected to produce a similar spectrum of haematological and clinical complications. However, it is clear that a marked variability characterizes this 'single gene' disorder and that many other genetic, environmental, and social factors determine the expression of SS disease. A greater understanding of these factors and their mechanism of operation may lead to new therapeutic approaches.

References

[1] F.B. Piel, A.P. Patil, R.E. Howes, O.A. Nyangiri, P.W. Gething, M. Dewi, W.H. Temperley, T.N. Williams, D.J. Weatherall, S.I. Hay, Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates, Lancet 381 (2013) 142–151.

[2] L. Pauling, H.A. Itano, S.J. Singer, I.C. Wells, Sickle cell anemia, a molecular disease, Science 110 (1949) 543–548.

[3] A.C. Allison, Malaria in carriers of the sickle-cell trait and in newborn children, Exp. Parasitol. 6 (1957) 418–447.

[4] J.B. Herrick, Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia, Arch. Intern. Med. 6 (1910) 517–521.

[5] T.L. Savitt, M.F. Goldberg, Herrick's 1910 case report of sickle cell anemia. The rest of the story, JAMA 261 (1989) 266–271.

[6] V.R. Mason, Sickle cell anemia, JAMA 79 (1922) 1318–1320.

[7] J.E. Cook, J. Meyer, Severe anemia with remarkable elongated and sickle-shaped red blood cells and chronic leg ulcers, Arch. Intern. Med. 16 (1915) 644–651.

[8] V.E. Emmel, A study of the erythrocytes in a case of severe anemia with elongated and sickle-shaped red blood corpuscles, Arch. Intern. Med. 20 (1917) 586–598.

[9] L.W. Diggs, C.F. Ahmann, J. Bibb, The incidence and significance of the sickle cell trait, Ann. Intern. Med. 7 (1933) 769–778.

[10] E.A. Beet, The genetics of the sickle-cell trait in a Bantu tribe, Ann. Eugenics 14 (1949) 279–284.

[11] J.V. Neel, The inheritance of sickle cell anemia, Science 110 (1949) 64–66.

[12] H. Lehmann, Sickle-cell anaemia and sickle-cell trait as homo- and heterozygous gene-combinations, Lancet i (1951) 1068.

[13] A.B. Raper, Sickle cell disease in Africa and America. A comparison, J. Trop. Med. Hyg. 53 (1950) 49–53.

[14] A.W.G. Teixeira, Hematias falciformes nos indigenas de Angola, Ann. Inst. Med. Trop. Lisboa 1 (1944) 365–374.

[15] H. Foy, A. Kondi, W. Brass, Sickle-cell disease of Africans in Kenya, East Afr. Med. J. 28 (1951) 1–5.

[16] J. Lambotte-Legrands, C. Lambotte-Legrands, L'anemie a hematies falciformes en Afrique Noire, Sang 23 (1952) 560–568.

[17] J.M. Vandepitte, Sickle-cell anaemia in the Belgian Congo, Trans. R. Soc. Trop. Med. Hyg. 46 (1952) 460–461.

[18] I.J. Sherman, The sickling phenomenon, with especial reference to the differentiationof sickle cell anemia from the sickle cell trait, Johns Hopkins Hosp. Bull. 67 (1940) 309–324.

[19] G.A. Daland, W.B. Castle, A simple and rapid method for demonstrating sickling of the red blood cells: the use of reducing agents, J. Lab. Clin. Med. 33 (1948) 1082–1088.

[20] J. Watson, A study of sickling of young erythrocytes in sickle cell anemia, Blood 3 (1948) 465–469.

[21] V.M. Ingram, A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin, Nature 178 (1956) 792–794.

[22] V.M. Ingram, Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin, Nature 180 (1957) 326–328.

[23] J.A. Hunt, V.M. Ingram, Allelomorphism and the chemical differences of the human haemoglobins A, S and C, Nature 181 (1958) 1062–1063.

[24] C.A. Marotta, J.T. Wilson, B.G. Forget, S.M. Weissman, Human β-globin messenger RNA III. Nucleotide sequences derived from complementary DNA, J. Biol. Chem. 252 (1977) 5040–5051.

[25] W.N. Powell, J.G. Rodarte, J.V. Neel, The occurrence in a family of Sicilian ancestry of the traits for both sickling and thalassemia, Blood 5 (1950) 887–897.

[26] E. Kaplan, W.W. Zuelzer, J.V. Neel, A new inherited abnormality of hemoglobin and its reaction with sickle cell hemoglobin, Blood 6 (1951) 1240–1259.

[27] P. Sturgeon, H.A. Itano, W.R. Bergren, Clinical manifestations of abnormal hemoglobins. 1. The interaction of hemoglobin-Swith hemoglobin-D, Blood 10 (1955) 389–404.

[28] R. Ramot, S. Fisher, D. Remex, R. Schneerson, D. Kahane, J.A.M. Ager, H. Lehmann, Haemoglobin O in an Arab family. Sickle cell-haemoglobin O Arab trait, Br. Med. J. 2 (1960) 1262–1264.

[29] E.J. Ahern, V.N. Ahern, G.H. Aarons, R.T. Jones, B. Brimhall, Hemoglobin Lepore Washington in two Jamaican families: interaction with beta chain variants, Blood 40 (1972) 246–256.

[30] F.B. Livingstone, Abnormal Hemoglobins in Human Populations, Aldine Publishing Company, Chicago, 1967.

[31] E.J. Hicks, G.D. Miller, R. Horton, Prevalence of sickle cell trait and HbC trait in Blacks from low socioeconomic conditions, Am. J. Public Health 68 (1978) 1135–1137.

[32] G.R. Serjeant, B.E. Serjeant, M. Forbes, R.J. Hayes, D.R. Higgs, H. Lehmann, Haemoglobin gene frequencies in the Jamaican population: a study of 100,000 newborns, Br. J. Haematol. 64 (1986) 253–262.

[33] P.I. Condon, R.J. Hayes, G.R. Serjeant, Retinal and choroidal neovascularization in sickle cell disease, Trans. Ophthalmol. Soc. U. K. 100 (1980) 434–439.

[34] J.C. Chang, Y.W. Kan, Beta 0 thalassemia, a nonsense mutation in man, Proc. Natl. Acad. Sci. U. S. A. 76 (1979) 2886–2889.

[35] N. Saha, B. Banerjee, Incidence of abnormal haemoglobins in Punjab, Calcutta Med. J. 62 (1965) 82–86.

[36] S.E. Antonarakis, C.D. Boehm, G.R. Serjeant, C.E. Theisen, G.J. Dover, H.H. Kazazian, Origin of the β^S-globin gene in Blacks: the contribution of recurrent mutation or gene conversion or both, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 853–856.

[37] J. Pagnier, J.G. Mears, O. Dunda-Belkhodja, K.E. Schaefer-Rego, C. Beldjord, R.L. Nagel, D. Labie, Evidence for the multicentric origin of the sickle cell hemoglobin gene in Africa, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 1771–1773.

[38] Y. Chebloune, J. Pagnier, G. Trabuchet, C. Faure, G. Verdier, D. Labie, V. Nigon, Structural analysis of the 5′ flanking region of the β-globin gene in African sickle cell anemia patients: further evidence for three origins of the sickle cell mutation in Africa, Proc. Natl. Acad. Sci. 85 (1988) 4431–4435.

[39] R.L. Nagel, The origin of the hemoglobin S gene: clinical, genetic, and anthropological consequences, Einstein Q. J. Biol. Med. 2 (1984) 53–62.

[40] C. Lapoumèroulie, O. Dunda, R. Ducrocq, G. Trabuchet, M. Mony-Lobé, J.M. Bodo, P. Carnevale, D. Labie, J. Elion, R. Krishnamoorthy, A novel sickle cell mutation of yet another origin in Africa: the Cameroon type, Hum. Genet. 89 (1992) 333–337.

[41] M.H. Steinberg, Z.-H. Lu, R.L. Nagel, S. Venkataramani, P.F. Milner, L. Huey, S. Safaya, R.F. Rieder, Hematological effects of atypical and Cameroon β-globin gene haplotypes in adult sickle cell anemia, Am. J. Hematol. 59 (1998) 121–126.

[42] N.S. Green, M.E. Fabry, L. Kaptue-Noche, R.L. Nagel, Senegal haplotype is associated with higher HbF than Benin and Cameroon haplotypes in African children with sickle cell anemia, Am. J. Hematol. 44 (1993) 145–146.

[43] D. Labie, J. Pagnier, C. Lapoumeroulie, F. Rouabhi, O. Dunda-Belkhodja, P. Chardin, C. Beldjord, H. Wajcman, M.E. Fabry, R.L. Nagel, Common haplotype dependency of high $^G\gamma$-globin gene expression and high HbF levels in β-thalassemia and sickle cell anemia patients, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 2111–2114.

[44] R.L. Nagel, M.E. Fabry, J. Pagnier, I. Zohoun, H. Wajcman, V. Baudin, D. Labie, Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type, N. Engl. J. Med. 312 (1985) 880–884.

[45] R.L. Nagel, S. Erlingsson, M.E. Fabry, H. Croizat, S.M. Susuka, H. Lachman, M. Sutton, C. Driscoll, E. Bouhassira, H.H. Billett, The Senegal DNA haplotype is associated with the amelioration of anemia in Africa-American sickle cell anemia patients, Blood 77 (1991) 1371–1375.

[46] P.F. Milner, J.G. Gilman, L. Huey, J.L. Wilson, R. Wrightstone, The significance of haplotype and alpha thalassemia in patients with sickle cell anemia in S.E. USA, Blood 74 (Suppl. 1) (1989) 260a.

[47] R.L. Nagel, S.K. Rao, O. Dunda-Belkohdja, M.M. Connolly, M.E. Fabry, A. Georges, R. Krishnamoorthy, D. Labie, The hematological characteristics of sickle cell anemia bearing the Bantu haplotype: the relationship between $^G\gamma$ and HbF level, Blood 69 (1987) 1026–1030.

[48] C. Oner, A.J. Dimovski, N.F. Olivieri, G. Schiliro, J.F. Codrington, S. Fattoum, A.D. Adekile, R. Oner, G.T. Yüregir, C. Altay, A. Gurgey, R.B. Gupta, V.B. Jogessar, M.N. Kitundu, D. Loukopoulos, G.P. Tamagnini, L.S. Ribeiro, F. Kutlar, L.-H. Gu, K.D. Lanclos, T.H.J. Huisman, β^S haplotypes in various world populations, Hum.

Genet. 89 (1992) 99–104.
[49] D.R. Powars, L. Chan, W.A. Schroeder, β^S-gene-cluster haplotypes in sickle cell anemia: clinical implications, Am. J. Pediatr. Hematol. Oncol. 12 (1990) 367–374.
[50] D.R. Powars, β^S-gene-cluster haplotypes in sickle cell anemia: clinical and hematologic features, Hematol. Oncol. Clin. North Am. 5 (1991) 475–493.
[51] D. Powars, A. Hiti, Sickle cell anemia. Beta s gene cluster haplotypes as genetic markers for severe disease expression, Am. J. Dis. Child. 147 (1993) 1197–1202.
[52] D.R. Powars, Sickle cell anemia and major organ failure, Hemoglobin 14 (1990) 573–598.
[53] D.R. Powars, H.J. Meiselman, T.C. Fisher, C. Johnson, Beta-S gene cluster haplotypes modulate hematologic and hemorheologic expression in sickle cell anemia. Use in predicting clinical severity, Am. J. Pediatr. Hematol. Oncol. 16 (1994) 55–61.
[54] R.F. Rieder, S. Safaya, P. Gillette, S. Fryd, H. Hsu, J.G. Adams, M.H. Steinberg, Effect of b-globin gene cluster haplotype on the haematological and clinical features of sickle cell anemia, Am. J. Hematol. 36 (1991) 184–189.
[55] S.K. Ballas, R.J.R. Castillo, The role of b-haplotypes in the clinical expression of sickle cell anemia, Am. J. Hematol. 39 (1992) 75 (letter).
[56] M. De Montalembert, M. Maier-Redelsperger, R. Girot, M. Belloy, E. Vilmer, R. Ducrocq, C. Guidal, J. Elion, β-globin gene cluster haplotype and α-thalassemia do not correlate with the acute clinical manifestations of sickle cell disease in children, Blood 82 (1993) 2595–2596.
[57] J.S. Wainscoat, S.L. Thein, D.R. Higgs, J.I. Bell, D.J. Weatherall, B.H. Al-Awamy, G.R. Serjeant, A genetic marker for elevated levels of haemoglobin F in homozygous sickle cell disease? Br. J. Haematol. 60 (1985) 261–268.
[58] B.C. Kar, R.K. Satapathy, A.E. Kulozik, M. Kulozik, S. Sirr, B.E. Serjeant, G.R. Serjeant, Sickle cell disease in Orissa State, India, Lancet ii (1986) 1198–1201.
[59] A.E. Kulozik, B.C. Kar, R.K. Satapathy, B.E. Serjeant, G.R. Serjeant, D.J. Weatherall, Fetal hemoglobin levels and β^s globin haplotypes in an Indian population with sickle cell disease, Blood 69 (1987) 1724–1726.
[60] D. Labie, R. Srinivas, O. Dunda, C. Dode, C. Lapoumeroulie, V. Devi, S. Devi, K. Ramasami, J. Elion, R. Ducrocq, R. Krisnamoorthy, R.L. Nagel, Haplotypes in tribal Indians bearing the sickle gene: evidence for the unicentric origin of the β^s mutation and the unicentric origin of the tribal populations of India, Hum. Biol. 61 (1989) 479–491.
[61] M.B. Mukherjee, R.R. Surve, R.R. Gangakhedkar, K. Ghosh, R.B. Colah, D. Mohanty, Beta-globin gene cluster haplotypes linked to the beta S gene in western India, Hemoglobin 28 (2004) 157–161.
[62] B. Bhagat, P.K. Patra, A.S. Thakur, Fetal haemoglobin and β-globin gene cluster haplotypes among sickle cell patients in Chhattisgarh, J. Clin. Diagn. Res. 7 (2013) 269–272.
[63] M.A. El-Hazmi, Beta-globin gene haplotypes in the Saudi sickle cell anaemia patients, Hum. Hered. 40 (1990) 177–186.
[64] A.E. Kulozik, J.S. Waiscoat, G.R. Serjeant, B.C. Kar, B. Al-Awamy, G.J.F. Esan, S.K. Hague, A.M. Hilali, S. Kate, W.A.E.P. Ranasinghe, D.J. Weatherall, Geographical survey of β^s-globin gene haplotypes: evidence for an independent Asian origin of the sickle-cell mutation, Am. J. Hum. Genet. 39 (1986) 239–244.
[65] M.A. Padmos, G.T. Roberts, K. Sackey, A. Kulozik, S. Bail, J.S. Morris, B.E. Serjeant, G.R. Serjeant, Two different forms of homozygous sickle cell disease occur in Saudi Arabia, Br. J. Haematol. 79 (1991) 93–98.
[66] A. Kutlar, Y. Hattori, I. Bakioglu, F. Kuttlar, K. Kamel, T.H.J. Huisman, Hematological observations on Arabian SS patients with a homozygosity or heterozygosity for a β^S chromosome with haplotype #31, Hemoglobin 9 (1985) 545–557.
[67] A.D. Adekile, L.H. Gu, E. Baysal, M.Z. Haider, L. al-Fuzae, K.C. Aboobacker, A. al-Rashied, T.H. Huisman, Molecular characterization of alpha-thalassemia determinants, beta-thalassemia alleles, and beta S haplotypes among Kuwaiti Arabs, Acta Haematol. 92 (1994) 176–181.
[68] F. Aghajani, M.R. Mahdavi, M. Kosaryan, M. Mahdavi, M. Hamidi, H. Jalali, Identification of β-globin haplotypes linked to sickle hemoglobin (Hb S) alleles in Mazandaran province, Iran, Genes Genet. Syst. (Dec 21 2016), http://dx.doi.org/10.1266/ggs.16-00005 (Epub ahead of print).
[69] B.A. Miller, M. Salameh, M. Ahmed, J. Wainscoat, G. Antognetti, S. Orkin, D. Weatherall, D.G. Nathan, High fetal hemoglobin production in sickle cell anemia in the eastern province of Saudi Arabia is genetically determined, Blood 67 (1986) 1404–1410.
[70] Y.C. Chang, K.D. Smith, R.D. Moore, G.R. Serjeant, G.J. Dover, An analysis of fetal hemoglobin variation in sickle cell disease: the relative contributions of the X-linked factor, β-globin haplotypes, α-globin gene number, gender and age, Blood 85 (1995) 1111–1117.
[71] A.P. Gelpi, Benign sickle cell disease in Saudi Arabia: survival estimates and population dynamics, Clin. Genet. 15 (1979) 307–310.
[72] R.P. Perrine, M.J. Brown, J.B. Clegg, D.J. Weatherall, A. May, Benign sickle-cell anaemia, Lancet ii (1972) 1163–1167.
[73] R.P. Perrine, M.E. Pembrey, P. John, S. Perrine, F. Shoup, Natural history of sickle cell disease in Saudi Arabs. A study of 270 subjects, Ann. Intern. Med. 88 (1978) 1–6.
[74] A.A. Mallouh, M.M. Salamah, Hypersplenism in homozygous sickle-cell disease in Saudi Arabia, Ann. Trop. Paediatr. 5 (1985) 143–146.
[75] D. Jain, V. Warthe, P. Diyama, D. Sarate, R. Colah, P. Mehta, G. Serjeant, Sickle cell disease in Central India: a potentially severe syndrome, Indian J. Pediatr. 83 (2016) 1071–1076.
[76] S.H. Embury, J.A. Miller, A.M. Dozy, Y.W. Kan, V. Chan, D. Todd, Two different molecular organizations account for the single α-globin gene of the α-thalassemia-2 genotype, J. Clin. Invest. 66 (1980) 1319–1325.
[77] I. Lubega, C.M. Ndugwa, E.A. Mworozi, J.K. Tumwine, Alpha thalassemia among sickle cell anaemia patients in Kampala, Uganda, Afr. Health Sci. 15 (2015) 682–689.
[78] C. Ndugwa, D. Higgs, I. Hambleton, K. Mason, B.E. Serjeant, G.R. Serjeant, Homozygous sickle cell disease in Uganda and Jamaica. A comparison of Bantu and Benin haplotypes, West Indian Med. J. 61 (2012) 684–691.
[79] M.B. Rumaney, V.J. Ngo Bitoungui, A.A. Vorster, R. Ramesar, A.P. Kengne, J. Ngogang, A. Wonkam, The co-inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients and could improve their survival, PLoS One 9 (2014) e100516.
[80] S.E. Cox, J. Makani, D. Soka, V.S. L'Esperence, E. Kija, P. Dominguez-Salas, C.R. Newton, A.A. Birch, A.M. Prentice, F.J. Kirkham, Haptoglobin, alpha-thalassaemia and glucose-6-phosphate dehydrogenase polymorphisms and risk of abnormal transcranial Doppler among patients with sickle cell anaemia in Tanzania, Br. J. Haematol. 165 (2014) 699–706.
[81] P. Purohit, S. Dehury, S. Patel, D.K. Patel, Prevalence of deletional alpha thalassemia and sickle gene in a tribal dominated malaria endemic area of eastern India, ISRN Hematol. 745245 (2014), http://dx.doi.org/10.1155/2014/745245 (eCollection 2014).
[82] M.B. Mukherjee, C.Y. Lu, R. Ducrocq, R.R. Gangakhedkar, R.B. Colah, M.D. Kadam, D. Mohanty, R.L. Nagel, R. Krishnamoorthy, Effect of alpha-thalassemia on sickle-cell anemia linked to the Arab-Indian haplotype in India, Am. J. Hematol. 55 (1997) 104–109.
[83] S.H. Embury, A.M. Dozy, J. Miller, J.R. David, K.M. Kleman, H. Preisler, E. Vichinsky, W.N. Lande, B.H. Lubin, Y.W. Kan, W.C. Mentzer, Concurrent sickle-cell anemia and α-thalassemia. Effect on severity of anemia, N. Engl. J. Med. 306 (1982) 270–274.
[84] D.R. Higgs, B.E. Aldridge, J. Lamb, J.B. Clegg, D.J. Weatherall, R.J. Hayes, Y. Grandison, Y. Lowrie, K.P. Mason, B.E. Serjeant, G.R. Serjeant, The interaction of alpha-thalassemia and homozygous sickle-cell disease, N. Engl. J. Med. 306 (1982) 1441–1446.
[85] K. De Ceulaer, D.R. Higgs, D.J. Weatherall, R.J. Hayes, B.E. Serjeant, G.R. Serjeant, α-thalassemia reduces the hemolytic rate in homozygous sickle-cell disease, N. Engl. J. Med. 309 (1983) 189–190.
[86] B.E. Serjeant, K.P. Mason, M.W. Kenny, J. Stuart, D. Higgs, D.J. Weatherall, R.J. Hayes, G.R. Serjeant, Effect of alpha thalassaemia on the rheology of homozygous sickle cell disease, Br. J. Haematol. 55 (1983) 479–486.
[87] S.H. Embury, M.R. Clark, G. Monroy, N. Mohandas, Concurrent sickle cell anemia and alpha-thalassemia. Effect on pathological properties of sickle erythrocytes, J. Clin. Invest. 73 (1984) 116–123.
[88] M.E. Fabry, J.G. Mears, P. Patel, K. Schaefer-Rego, L.D. Carmichael, G. Martinez, R.L. Nagel, Dense cells in sickle cell anemia: the effects of gene interaction, Blood 64 (1984) 1042–1046.
[89] R.J. Adams, A. Kutlar, V. McKie, E. Carl, F.T. Nichols, J.C. Liu, K. McKie, A. Clary, Alpha thalassemia and stroke risk in sickle cell anemia, Am. J. Hematol. 45 (1994) 279–282.
[90] F.M. Gill, A. Sleeper, S.J. Weiner, A.K. Brown, R. Bellevue, R. Grover, C.H. Pegelow, Vichinsky E and the cooperative study of sickle cell disease, Blood 86 (1995) 776–783.
[91] B. Balkaran, G. Char, J.S. Morris, B.E. Serjeant, G.R. Serjeant, Stroke in a cohort study of patients with homozygous sickle cell disease, J. Pediatr. 120 (1992) 360–366.
[92] S.T. Miller, R.F. Rieder, S.P. Rao, A.K. Brown, Cerebrovascular accidents in children with sickle-cell disease and alpha-thalassemia, J. Pediatr. 113 (1988) 847–849.
[93] K. Ohene-Frempong, S.J. Weiner, L.A. Sleeper, S.T. Miller, S. Embury, J.W. Moohr, D.L. Wethers, C.H. Pegelow, F.M. Gill, Cooperative Study of Sickle Cell Disease, Cerebrovascular accidents in sickle cell disease: rates and risk factors, Blood 91 (1998) 288–294.
[94] D. Nebor, C. Broquere, K. Brudey, D. Mougenel, V. Tarer, P. Connes, J. Elion, M. Romana, Alpha-thalassemia is associated with a decreased occurrence and a delayed age-at-onset of albuminuria in sickle cell anemia patients, Blood Cells Mol. Dis. 45 (2010) 154–158.
[95] A. Wonkam, M.B. Rumaney, V.J. Ngo Bitoungui, A.A. Vorster, R. Ramesar, J. Ngogang, Coinheritance of sickle cell anemia and α-thalassemia delays disease onset and could improve survival in Cameroonian's patients (Sub-Saharan Africa), Am. J. Hematol. 89 (2014) 664–665.
[96] G. Martinez, A. Muniz, E. Svarch, E. Espinosa, R.L. Nagel, Age dependence of the gene frequency of alpha-thalassemia in sickle cell anemia in Cuba, Blood 88 (1996) 1898–1899.
[97] J.G. Mears, H.M. Lachman, D. Labie, R.L. Nagel, Alpha-thalassemia is related to prolonged survival in sickle cell anemia, Blood 62 (1983) 286–290.
[98] L. Kéclard, M. Romana, E. Lavocat, C. Saint-Martin, C. Berchel, G. Mérault, Sickle cell disorder, β-globin gene cluster haplotypes and α-thalassemia in neonates and adults from Guadeloupe, Am. J. Hematol. 55 (1997) 24–27.
[99] S.K. Ballas, C.A. Talacki, V.M. Rao, R.M. Steiner, The prevalence of avascular necrosis in sickle cell anemia: correlation with α-thalassemia, Hemoglobin 13 (1989) 649–655.
[100] M.H. Steinberg, W. Rosenstock, M.B. Coleman, J.G. Adams, O. Platica, M. Cedeno, R.F. Rieder, J.T. Wilson, P. Milner, S. West, Effects of thalassemia and microcytosis on the hematologic and vaso-occlusive severity of sickle cell anemia, Blood 63 (1984) 1353–1360.
[101] H.H. Billett, K. Kim, M.E. Fabry, R.L. Nagel, The percentage of dense red cells does not predict incidence of sickle cell painful crisis, Blood 68 (1986) 301–303.
[102] H.H. Billett, M.E. Fabry, R.L. Nagel, Paradoxical increase of painful crises in sickle

cell patients with α-thalassemia, Blood 86 (1995) 4382 (letter).
[103] S. Bailey, D.R. Higgs, J. Morris, G.R. Serjeant, Is the painful crisis of sickle cell disease due to sickling? Lancet 337 (1991) 735 (letter).
[104] D.S. Darbari, O. Onyekwere, M. Nouraie, C.P. Minniti, L. Luchtman-Jones, S. Rana, C. Sable, G. Ensing, N. Dham, A. Campbell, M. Arteta, M.T. Gladwin, O. Castro, J.G. Taylor, G.J. Kato, V. Gordeuk, Markers of severe vaso-occlusive painful episode frequency in children and adolescents with sickle cell anemia, J. Pediatr. 160 (2012) 286–290.
[105] P. Joly, C. Pondarré, C. Bardel, A. Francina, C. Martin, The alpha-globin genotype does not influence sickle cell disease severity in a retrospective cross-validation study of the pediatric severity score, Eur. J. Haematol. 88 (2012) 61–67.
[106] S.K. Ballas, Effect of α-globin genotype on the pathophysiology of sickle cell disease, Pediatr. Pathol. Mol. Med. 20 (2001) 107–121.
[107] K.P. Mason, Y. Grandison, R.J. Hayes, B.E. Serjeant, G.R. Serjeant, S. Vaidya, W.G. Wood, Post-natal decline of fetal haemoglobin in homozygous sickle cell disease: relationship to parental HbF levels, Br. J. Haematol. 52 (1982) 455–463.
[108] R. Bainbridge, D.R. Higgs, G.H. Maude, G.R. Serjeant, Clinical presentation of homozygous sickle cell disease, J. Pediatr. 106 (1985) 881–885.
[109] G.R. Serjeant, Fetal haemoglobin in homozygous sickle cell disease, Clin. Haematol. 4 (1975) 109–122.
[110] R.J. Hayes, M. Beckford, Y. Grandison, K. Mason, B.E. Serjeant, G.R. Serjeant, The haematology of steady state homozygous sickle cell disease. Frequency distributions, variation with age and sex, longitudinal observations, Br. J. Haematol. 59 (1985) 369–382.
[111] H.T. Bae, C.T. Baldwin, P. Sebastiani, M.J. Telen, A. Ashley-Koch, M. Garrett, W.C. Hooper, C.J. Bean, M.R. Debaun, D.E. Arking, P. Bhatnagar, J.F. Casella, J.R. Keefer, E. Barron-Casella, V. Gordeuk, G.J. Kato, C. Minniti, J. Taylor, A. Campbell, L. Luchtman-Jones, C. Hoppe, M.T. Gladwin, Y. Zhang, M.H. Steinberg, Meta-analysis of 2040 sickle cell anemia patients: BCL11A and HBS1L-MYB are the major modifiers of HbF in African Americans, Blood 120 (2012) 1961–1962.
[112] A.A. Bhanushali, P.K. Patra, D. Nair, H. Verma, B.R. Das, Genetic variant in the BCL11A (rs1427407), but not HBS1-MYB (rs6934903) loci associate with fetal hemoglobin levels in Indian sickle cell disease patients, Blood Cells Mol. Dis. 54 (2015) 4–8.
[113] G. Lettre, V.G. Sankaran, M.A. Bezerra, A.S. Araujo, M. Uda, S. Sanna, A. Cao, D. Schlessinger, F.F. Costa, J.N. Hirschhorn, S.H. Orkin, DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11869–11874.
[114] A.E. Sedgewick, N. Timofeev, P. Sebastiani, J.C. So, E.S. Ma, L.C. Chan, G. Fucharoen, S. Fucharoen, C.G. Barbosa, B.N. Vardarajan, L.A. Farrer, C.T. Baldwin, M.H. Steinberg, D.H. Chui, BCL11A is a major HbF quantitative trait locus in three different populations with beta-hemoglobinopathies, Blood Cells Mol. Dis. 41 (2008) 255–258.
[115] D.E. Bauer, S.C. Kamran, S.H. Orkin, Reawakening fetal hemoglobin: prospects for new therapies for the beta-globin disorders, Blood 120 (2012) 2945–2953.
[116] J. Borg, P. Papadopoulos, M. Georgitsi, L. Gutiérrez, G. Grech, P. Fanis, M. Phylactides, A.J. Verkerk, P.J. van der Spek, C.A. Scerri, W. Cassar, R. Galdies, W. van Ijcken, Z. Ozgür, N. Gillemans, J. Hou, M. Bugeja, F.G. Grosveld, M. von Lindern, A.E. Felice, G.P. Patrinos, S. Philipsen, Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin, Nat. Genet. 42 (2010) 801–805, http://dx.doi.org/10.1038/ng.630 (Epub 2010 Aug 1).
[117] W. Deng, J. Lee, H. Wang, J. Miller, A. Reik, P.D. Gregory, A. Dean, G.A. Blobel, Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor, Cell 149 (2012) 1233–1244.
[118] G. Galarneau, C.D. Palmer, Orkin S.H. SankaranVG, J.N. Hirschhorn, G. Lettre, Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation, Nat. Genet. 42 (2010) 1049–1051.
[119] J. Makani, S. Menzel, S. Nkya, S.E. Cox, E. Drasar, D. Soka, A.N. Komba, J. Mgaya, H. Rooks, H.N. Vasavda, G. Fegan, C.R. Newton, M. Farrall, S.L. Thein, Genetics of fetal hemoglobin in Tanzanian and British patients with sickle cell anemia, Blood 117 (2011) 1390–1392.
[120] S.N. Mtatiro, T. Singh, H. Rooks, J. Mgaya, H. Mariki, D. Soka, B. Mmbando, E. Msaki, I. Kolder, S.L. Thein, S. Menzel, S.E. Cox, J. Makani, J.C. Barrett, Genome wide association study of fetal hemoglobin in sickle cell anemia in Tanzania, PLoS One 9 (2014) e111464.
[121] D. Ngo, H. Bae, M.H. Steinberg, P. Sebastiani, N. Solovieff, C.T. Baldwin, E. Melista, S. Safaya, L.A. Farrer, A.M. Al-Suliman, W.H. Albuali, M.H. Al Bagshi, Z. Naserullah, I. Akinsheye, P. Gallagher, H.Y. Luo, D.H. Chui, J.J. Farrell, A.K. Al-Ali, A. Alsultan, Fetal hemoglobin in sickle cell anemia: genetic studies of the Arab-Indian haplotype, Blood Cells Mol. Dis. 51 (2013) 22–26.
[122] V.G. Sankaran, S.H. Orkin, The switch from fetal to adult hemoglobin, Cold Spring Harb. Perspect. Med. 3 (2013) a011643.
[123] N. Solovieff, J.N. Milton, S.W. Hartley, R. Sherva, P. Sebastiani, D.A. Dworkis, E.S. Klings, L.A. Farrer, M.E. Garrett, A. Ashley-Koch, M.J. Telen, S. Fucharoen, S.Y. Ha, C.K. Li, D.H. Chui, C.T. Baldwin, M.H. Steinberg, Fetal hemoglobin in sickle cell anemia: genome-wide association studies suggest a regulatory region in the 5′ olfactory receptor gene cluster, Blood 115 (2010) 1815–1822.
[124] M.H. Steinberg, P. Sebastiani, Genetic modifiers of sickle cell disease, Am. J. Hematol. 87 (2012) 795–803.
[125] D. Zhou, K. Liu, C.W. Sun, K.M. Pawlik, T.M. Townes, KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching, Nat. Genet. 42 (2010) 742–744, http://dx.doi.org/10.1038/ng.637 (Epub 2010 Aug 1).
[126] W. Deng, J.W. Rupon, I. Krivega, L. Breda, I. Motta, K.S. Jahn, A. Reik, P.D. Gregory, S. Rivella, A. Dean, G.A. Blobel, Reactivation of developmentally silenced globin genes by forced chromatin looping, Cell 158 (2014) 849–860.
[127] G.J. Kato, M.T. Gladwin, M.H. Steinberg, Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes, Blood Rev. 21 (2007) 37–47.
[128] F.S. Collins, C.J. Stoeckert Jr., G.R. Serjeant, B.G. Forget, S.M. Weissman, G gamma beta + hereditary persistence of fetal hemoglobin: cosmid cloning and identification of a specific mutation 5′ to the G gamma gene, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 4894–4898.
[129] I. Akinsheye, A. Alsultan, N. Solovieff, D. Ngo, C.T. Baldwin, P. Sebastiani, D.H.K. Chui, M.H. Steinberg, Fetal hemoglobin in sickle cell anemia, Blood 118 (2011) 19–27.
[130] J.E. MacIver, E.J. Parker-Williams, The aplastic crisis in sickle cell anaemia, Lancet i (1961) 1086–1089.
[131] G.H. Maude, R.J. Hayes, G.R. Serjeant, The haematology of steady state homozygous sickle cell disease: interrelationships between haematological indices, Br. J. Haematol. 66 (1987) 549–558.
[132] M.C.G. Stevens, R.J. Hayes, S. Vaidya, G.R. Serjeant, Fetal hemoglobin and clinical severity of homozygous sickle cell disease in early childhood, J. Pediatr. 98 (1981) 37–41.
[133] K. Bailey, J.S. Morris, G.R. Serjeant, Fetal haemoglobin and early manifestations of homozygous sickle cell disease, Arch. Dis. Child. 67 (1992) 517–520.
[134] G.R. Serjeant, Irreversibly sickled cells and splenomegaly in sickle-cell anaemia, Br. J. Haematol. 19 (1970) 635–641.
[135] B. Al-Awamy, W.A. Wilson, H.A. Pearson, Splenic function in sickle cell disease in the Eastern Province of Saudi Arabia, J. Pediatr. 104 (1984) 714–717.
[136] D. Jain, A.S. Bagal, M. Shah, V. Sarathi, Morbidity patterns in hospitalized under five children with sickle cell disease, Indian J. Med. Res. 138 (2013) 317–321.
[137] G.R. Serjeant, K. Ghosh, J. Patel, Sickle cell disease in India: a perspective. Editorial, Indian J. Med. Res. 143 (2016) 102–105.
[138] G.R. Serjeant, The challenge of Indian sickle cell disease: evolving locally appropriate models of care, Indian J. Med. Res. 143 (2016) 405–413.
[139] H.S. Zarkowsky, D. Gallagher, F.M. Gill, W.C. Wang, J.M. Falletta, W.M. Lande, P.S. Levy, J.I. Verter, D. Wethers, Cooperative Study of Sickle Cell Disease, Bacteremia in sickle hemoglobinopathies, J. Pediatr. 109 (1986) 579–585.
[140] B.C. Kar, Sickle cell disease in India, J. Assoc. Physicians India 39 (1991) 954–960.
[141] M.C.G. Stevens, M. Padwick, G.R. Serjeant, Observations on the natural history of dactylitis in homozygous sickle cell disease, Clin. Pediatr. 20 (1981) 311–317.
[142] K.F. Baum, D.T. Dunn, G.H. Maude, G.R. Serjeant, The painful crisis of homozygous sickle cell disease: a study of risk factors, Arch. Intern. Med. 147 (1987) 1231–1234.
[143] O.S. Platt, B.D. Thorington, D.J. Brambilla, P.F. Milner, W.F. Rosse, E. Vichinsky, T.R. Kinney, Pain in sickle cell disease. Rates and risk factors, N. Engl. J. Med. 325 (1991) 11–16.
[144] G.R. Serjeant, C. De Ceulaer, R. Lethbridge, J.S. Morris, A. Singhal, P.W. Thomas, The painful crisis of homozygous sickle cell disease - clinical features, Br. J. Haematol. 87 (1994) 586–591.
[145] J.S. Mohan, J.M. Marshall, H.L. Reid, P.W. Thomas, G.R. Serjeant, Peripheral vascular response to mild indirect cooling in patients with homozygous sickle cell (SS) disease and the frequency of painful crisis, Clin. Sci. 94 (1998) 111–120.
[146] G.R. Serjeant, R.M. Chalmers, Is the painful crisis of sickle cell disease a “steal” syndrome? J. Clin. Pathol. 43 (1990) 789–791.
[147] D.R. Powars, J.N. Weiss, L.S. Chan, W.A. Schroeder, Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? Blood 63 (1984) 921–926.
[148] A. Donaldson, P. Thomas, B.E. Serjeant, G.R. Serjeant, Foetal haemoglobin in homozygous sickle cell disease: a study of patients with low HbF levels, Clin. Lab. Haematol. 23 (2001) 285–289.
[149] S.R. Goodman, B.S. Pace, K.C. Hansen, A. D'Alessandro, Y. Xia, O. Daescu, S.J. Glatt, Minireview: multiomic candidate biomarkers for clinical manifestations of sickle cell severity: early steps to precision medicine, Exp. Biol. Med. (Maywood) 241 (2016) 772–781.
[150] B.W. Hounkpe, M.M. Fiusa, M.P. Colella, L.N. da Costa, O. Benatti Rde, S.T. Saad, F.F. Costa, M.N. dos Santos, E.V. De Paula, Role of innate immunity-triggered pathways in the pathogenesis of Sickle Cell Disease: a meta-analysis of gene expression studies, Sci Rep 5 (2015) 17822.
[151] A. Habara, M.H. Steinberg, Minireview: genetic basis of heterogeneity and severity in sickle cell disease, Exp. Biol. Med. (Maywood) 241 (2016) 689–696.
[152] A. Basu, S. Saha, S. Karmakar, S. Chakravarty, D. Banerjee, B.P. Dash, A. Chakrabarti, 2D DIGE based proteomics study of erythrocyte cytosol in sickle cell disease: altered proteostasis and oxidative stress, Proteomics 13 (2013) 3233–3242, http://dx.doi.org/10.1002/pmic.201300177 (Epub 2013 Oct 1).
[153] A. Hryniewicz-Jankowska, P.K. Choudhary, L.P. Ammann, C.T. Quinn, S.R. Goodman, Monocyte protein signatures of disease severity in sickle cell anemia, Exp. Biol. Med. (Maywood) 234 (2009) 210–221.
[154] D.G. Kakhniashvili, N.B. Griko, L.A. Bulla, S.R. Goodman, The proteomics of sickle cell disease: profiling of erythrocyte membrane proteins by 2D-DIGE and tandem mass spectrometry, Exp. Biol. Med. (Maywood) 230 (2005) 787–792.
[155] A. Tumblin, A. Tailor, G.T. Hoehn, A.K. Mack, L. Mendelsohn, L. Freeman, X. Xu, A.T. Remaley, P.J. Munson, A.F. Suffredini, G.J. Kato, Apolipoprotein A-I and serum amyloid A plasma levels are biomarkers of acute painful episodes in patients with sickle cell disease, Haematologica 95 (2010) 1467–1472.
[156] S. Yuditskaya, A. Tumblin, G.T. Hoehn, G. Wang, S.K. Drake, X. Xu, S. Ying, A.H. Chi, A.T. Remaley, R.F. Shen, P.J. Munson, A.F. Suffredini, G.J. Kato, Proteomic identification of altered apolipoprotein patterns in pulmonary hypertension and vasculopathy of sickle cell disease, Blood 113 (2009) 1122–1128.

[157] D. Darghouth, B. Koehl, C. Junot, P.H. Romeo, Metabolomic analysis of normal and sickle cell erythrocytes, Transfus. Clin. Biol. 17 (2010) 148–150.
[158] D. Darghouth, B. Koehl, G. Madalinski, J.F. Heilier, P. Bovee, Y. Xu, M.F. Olivier, P. Bartolucci, M. Benkerrou, S. Pissard, Y. Colin, F. Galacteros, G. Bosman, C. Junot, P.H. Romeo, Pathophysiology of sickle cell disease is mirrored by the red blood cell metabolome, Blood 117 (2011) e57–e66.
[159] C.R. Morris, Alterations of the arginine metabolome in sickle cell disease: a growing rationale for arginine therapy, Hematol. Oncol. Clin. North Am. 28 (2014) 301–321.
[160] Y. Zhang, Y. Dai, J. Wen, W. Zhang, A. Grenz, H. Sun, L. Tao, G. Lu, D.C. Alexander, M.V. Milburn, L. Carter-Dawson, D.E. Lewis, W. Zhang, H.K. Eltzschig, R.E. Kellems, M.R. Blackburn, H.S. Juneja, Y. Xia, Detrimental effects of adenosine signaling in sickle cell disease, Nat. Med. 17 (2011) 79–86.
[161] M.W. Weatherall, D.R. Higgs, H. Weiss, D.J. Weatherall, G.R. Serjeant, Genotype/phenotype relationships in sickle cell disease: a pilot twin study, Clin. Lab. Haematol. 27 (2005) 384–390.
[162] F.B. Piel, S. Tewari, V. Brousse, A. Analitis, A. Font, S. Menzel, S. Chakravorty, S.L. Thein, B. Inusa, P. Telfer, M. de Montalembert, G.W. Fuller, K. Katsouyanni, D.C. Rees, Associations between environmental factors and hospital admissions for sickle cell disease, Haematologica (Dec 1 2016) (pii: haematol.2016.154245. [Epub ahead of print]).
[163] S. Tewari, V. Brousse, F.B. Piel, S. Menzel, D.C. Rees, Environmental determinants of severity in sickle cell disease, Haematologica 100 (2015) 1108–1116.