Sickle Cell Disease

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Abstract

Sickle cell disease (SCD) is a group of inherited disorders caused by mutations in *HBB*, which encodes the β-globin chain of haemoglobin. The incidence is estimated between 300,000 and 400,000 neonates globally each year, the majority in sub-Saharan Africa. Haemoglobin molecules that include sickle β-globin chains can polymerize; erythrocytes that contain mostly haemoglobin polymers assume a sickled form and are prone to haemolysis. Other pathophysiological mechanisms that contribute to SCD phenotype are vaso-occlusion and activation of the immune system. SCD is characterized by a remarkable phenotypic complexity. Common acute complications are acute pain events, acute chest syndrome and stroke; chronic complications (including chronic kidney disease) can damage all organs. Hydroxycarbamide, blood transfusions and haematopoietic stem cell transplantation can reduce the severity of the disease. Early diagnosis is crucial to improve survival and universal newborn babies screening programmes have been implemented in some countries, but are challenging in low-income, high-burden settings.

[H1] Introduction

Sickle cell disease (SCD) is an umbrella term that defines a group of inherited diseases (including SCA, HbSC and HbSβ-thalassaemia, see below) characterized by mutations in the gene encoding the haemoglobin subunit β (*HBB*) (Figure **1**). Haemoglobin (Hb) is a tetrameric protein composed of different combinations of globin subunits; each globin subunit is associated with the cofactor heme, which can carry a molecule of oxygen. Hb is expressed by red blood cells, both reticulocytes (immature red blood cells) and erythrocytes (mature red blood cells). Several genes encode different types of globin proteins, and their various tetrameric combinations generate multiple types of Hb, which are normally expressed at different stages of life —embryonic, foetal and adult. HbA, the most abundant (>90%) form of adult Hb, comprises two α globin subunits (encoded by the duplicated *HBA1* and *HBA2* genes) and two β-globin subunits. A mutation in *HBB* that causes an amino acid substitution in the β globin protein results in the sickle Hb (HbS) allele β^S. Under conditions of deoxygenation (that is, when the Hb is not bound to oxygen (O_2)) Hb tetramers that include two of these mutant sickle β globin subunits (that is, HbS) can polymerize and cause the erythrocytes to assume a crescent or sickled shape from which the disease takes its name. Hb tetramers with one HbS subunit can also polymerize, albeit not as efficiently as tetramers with two HbS subunits. Sickle erythrocytes can lead to recurrent vaso-occlusive episodes that are the hallmark of SCD.

SCD is inherited as an autosomal codominant trait[1]; individuals who are heterozygous for the β^S allele carry the sickle cell trait (HbAS) but do not have SCD, whereas individuals who are homozygous for β^S allele have sickle cell anaemia (SCA). SCA, the most common form of SCD, is a lifelong disease characterized by chronic haemolytic anaemia, unpredictable episodes of pain and widespread organ damage. There is a wide variability in the clinical severity of SCA, as well as in the life expectancy[2]. Genetic and genome-wide association studies have consistently found that high levels of foetal haemoglobin (HbF; the heterodimeric combination of two α-globin proteins and two γ-globin proteins (encoded by *HBG1* and *HBG2*)[3] and the co-inheritance of α thalassaemia (which is caused by mutations in *HBA1* and *HBA2*) are associated on average with milder SCD phenotypes[2]. However, these two biomarkers only explain a small fraction of the observed phenotypic variability.

Since the 1980's, a rapidly expanding body of knowledge has promoted a better understanding of SCD, particularly in high-income countries[4,5]. In the United States, research funding increased exponentially, awareness and education programmes expanded, counselling programmes were improved and universal newborn screening programmes now ensure early diagnosis and intervention. Specific research and training programmes led to a cadre of knowledgeable health professionals working in this field, improved patient management, prevention of complications and extension of life expectancy.
In this Primer, we will focus on SCA and aim to balance such remarkable advances with the key major challenges remaining worldwide to improve the prevention and management of this chronic disease, and ultimately to discover an affordable cure.

[H1] Epidemiology

[H2] NATURAL HISTORY

There is relatively little information on the natural history of the disease (which is relevant for SCD prevention and control), especially in areas of high prevalence. The main sources of information are the Jamaican Cohort Study of Sickle Cell Disease, which initiated in 1973 and followed up all cases of SCD detected among 100,000 consecutive deliveries in Kingston, Jamaica[6], and, in the United States, the Cooperative Study of Sickle Cell Disease (CSSCD, 1978–1998), which gathered data on growth and development, disease complications, clinical studies and epidemiological data on >3,000 patients with SCD[7]. Since the discontinuation of the CSSCD, the ongoing natural history of SCD in the United States can be gleaned from a few single-institution ongoing registries, screening populations of clinical trial cohorts and administrative health data sets.

Several cohort studies in high-income and middle-income countries have demonstrated that the clinical course of SCD has substantially changed since the 1970's in both children and adults. Survival similar to that of healthy children have been reported in children with SCA in the United States and the United Kingdom[8]. Adults with SCD in high-income countries can now expect to live well into their 60s and a median survival of 67 years has been reported for patients with SCD at one London hospital[9]; nevertheless, survival is still much lower than that of the general population of London. As childhood mortality of SCD has fallen, the transition from paediatric to adult patterns of lifestyle and medical care delivery is increasingly important. For example, in the United States there is a declining workforce of adult haematologists who are trained specifically in SCD, which means that adults with SCD are treated by primary care physicians or by haematologists-oncologists who are minimally experienced in SCD. There are limited data available about the survival of patients with SCD in sub-Saharan Africa and India. Data from African studies indicate childhood SCA mortality (before 5 years of age) of 50–90%[10].

[H2] DISTRIBUTION

The geographic distribution of β^S allele is mainly driven by two factors: the endemicity of malaria and population movements. The overlap between the geographical distribution of the β^S allele and malaria endemicity in Sub-Saharan Africa led in the 1950s to the hypothesis that individuals with HbAS might benefit from a protection against *Plasmodium falciparum* malaria[11]. There is now clear evidence that HbAS provides a remarkable protection against severe *P. falciparum* malaria[12] (in fact, individuals with HbAS are 90% less likely to experience severe malaria than individuals with only normal Hb), which explains the high frequencies of the β^S allele observed across Sub-Saharan Africa and parts of the Mediterranean, the Middle East and India[13]. Population movements, including the slave trade, have led to a much wider distribution of β^S allele, particularly in North America and Western Europe[14]. Detailed mapping of β^S allele frequency has highlighted that geographic heterogeneities in the prevalence of inherited haemoglobin disorders can occur over short distances[15].

[H2] PREVALENCE and INCIDENCE

The incidence of SCA births in sub-Saharan Africa has been estimated to ~230,000 in 2010, which corresponds to ~75% of births with SCA worldwide (**Figure 2**) [14]. In addition, West Africa has the highest incidence of HbSC disease, the second most common type of SCD (**Figure 1**)[16]. Over the next 40 years, these numbers are predicted to increase, particularly in sub-Saharan Africa[17]. The 2010 estimates reported >3.5 million newborn infants with HbAS in sub-Saharan Africa, who could benefit from a potent

protection from severe *P. falciparum* malaria and associated mortality[13]. To date, no African country has implemented a national screening programme for SCD[18]. Even in countries where universal screening programmes have been in place for >10 years (for example, the United Kingdom), estimating prevalence, incidence and burden of disease remains challenging[19,20]. In the last 20 years, ~40,000 confirmed cases of SCD were identified in 76 million newborn babies, with >1.1 million newborn babies with HbAS genotype in the United States[21]. Thus, 1 in every 1,941 neonates has SCD, and 1 in every 67 was heterozygous for the β^S allele.

The incidence of SCD varies by state, race and ethnicity[22,23]. Among African-Americans, ~1 in 360 newborn babies have SCD. Substantial demographic changes have resulted in a more-diverse population at risk and a high prevalence of SCD in immigrant populations. New-born babies screening studies for SCD in New York State document the marked effect of immigration on the frequency of neonates with SCD[24], as most of them have foreign-born mothers.

The incidence of SCD in newborn babies varies substantially among the states in Brazil, reflecting the ethnic heterogeneity of the Brazilian population. In 2014 the incidence of SCD was ~1 in 650 newborn babies screened in the state of Bahia, 1 in 1,300 in the state of Rio de Janeiro and 1 in 13,500 in the state of Santa Catarina[25]. Nationwide, in 2016, 1,071 newborn babies had SCD and >60,000 were heterozygotes for the β^S allele[26]. There are an estimated 30,000 patients with SCD in the whole country. The prevalence of β^S allele in Brazil varies from 1.2% to 10.9%, depending on the region, whereas the prevalence of β^C allele is reported between 0.15% and 7.4%.[27-29] The number of all-age individuals affected by SCA globally is currently unknown and cannot be estimated reliably owing to the paucity of epidemiological data, in particular mortality data, in areas of high prevalence.

[H2] DISEASE SEVERITY

The variability in the clinical severity of SCA can partly be explained by genetic modifiers, including HbF level and co-inheritance of α-thalassaemia (see below)[30,31]. For example, the Arab-India haplotype (a haplotype is a set of DNA polymorphisms that are inherited together) that is found in an area extending from the eastern coast of Saudi Arabia and East Africa to India) is considered to be associated with a phenotype milder than the four African haplotypes (Benin, Bantu, Cameroon and Senegal haplotypes) and, within India, this phenotype could be milder in the tribal populations than in the non-tribal populations[32], owing to a higher level of HbF[31]. However, evidence suggests that the range of severity of SCD in India might be wider than previously thought[33]. Environmental factors (such as the home environment, socio-economic status, nutrition and access to care) also influence the severity of the disease but, apart from malaria, their role has rarely been investigated[34,35]. Although some complications are more frequent in some regions than in others (for example, leg ulcers are common in tropical regions but relatively rare in temperate climates[36], whereas priapism is common in patients of African ancestry but rarer in those of Indian ancestry[37]), these geographical differences have never been comprehensively and rigorously documented.

[H2] DISEASE BURDEN

It has been estimated that 50-90% of children with SCA who live in sub-Saharan Africa die by 5 years of age[10]. Most of these children die from infections – invasive pneumococcal disease and malaria[38,39]. Owing to the limited data across most areas of high-prevalence, it is difficult to precisely assess the future health and economic burden of SCD. As low-income and middle-income countries go through the epidemiologic transition (that is, changing patterns of population age distributions, mortality, fertility, life expectancy and causes of death, largely driven by public health improvements), which involves substantial reductions in infant mortality that allow for SCA diagnoses and treatment, and international migrations contribute to further expand the distribution of the β^S allele, the health burden of this disease will increase[40]. Demographic projections estimated that the annual number of newborn babies with SCA worldwide will reach > 400,000 by 2050[17].

[H1] Mechanisms/pathophysiology

The landmark complication associated with SCA is the vaso-occlusive painful crisis. Although vaso-occlusion is a complex phenomenon, HbS polymerization is the essential pathophysiological occurrence in SCA[41-43]. HbS polymerization changes the shape and physical properties of erythrocytes, resulting in haemolytic anaemia and blockage of blood flow, particularly in small (and some large) vessels, that can damage any organ. HbS polymerization can also occur in reticulocytes, which account for ~20% of the red blood cells in patients with SCA. Direct and indirect consequences of haemolysis play a part in modifying the course and complications of SCD. Furthermore, HbS polymers lead to other abnormalities at the cellular level that contribute to the overall pathophysiological mechanism of SCD. The pathophysiology of the several variant genotypes of SCD (double heterozygous states or SCA with modifying genes) share the common pathophysiology as described in this section. The variants provide nuanced phenotypic differences or reduced severity (Figure 1).

[H2] ERYTHROCYTE MORPHOLOGY

[H3] HbS oxygen affinity and polymerization. HbS has reduced oxygen affinity compared with HbA. Reduced HbS oxygen affinity exacerbates HbS polymerization, which in turn further reduces HbS oxygen affinity[44] (Figure 3). HbS oxygen affinity is further reduced by 2,3-diphosphoglycerate (2,3-DPG), which is a glycolytic intermediate that is physiologically present at very high levels in sickle erythrocytes and, through interaction with deoxygenated β globin subunits, reduces Hb oxygen affinity[45]. At any partial pressure of oxygen (pO_2), low HbS oxygen affinity kinetically favours an increase in the fraction of deoxygenated HbS, (which is the tense conformation (T-state) that readily polymerizes), which in turn promotes HbS polymerization and the formation of sickle erythrocytes. Initial reports indicate that sickle erythrocytes have increased sphingosine kinase activity, which leads to high levels of sphingosine-1-phosphate, which also decreases HbS oxygen affinity[46]. Sphingosine kinase is activated by increased levels of plasma adenosine (which result from the hydrolysis of adenosine nucleotides that are released from erythrocytes during haemolysis) via the erythrocyte adenosine receptor A2b[47,48].

HbS polymerization correlates exponentially with the concentration of HbS within the erythrocyte, and also with the composition of other hemoglobins that variably participate in polymers **[JA: please add here this NEW ref:** Noguchi, C. T. & Schechter, A. N. Sickle hemoglobin polymerization in solution and in

cells. *Annual review of biophysics and biophysical chemistry* **14**, 239-263, (1985) **and add it to the bibliography too]**. In α-thalassaemia, reduced production of α globin subunits favours the formation of unstable β^s tetramers (formed by four sickle β globin subunits) which are proteolyzed, leaving a lower HbS concentration, which slows HbS polymerization and haemolysis. Abnormal cation homeostasis (described in the following section) in sickle erythrocytes leads to cell dehydration, which results in increased HbS concentration and polymerization (Figure 3)[49]. As the polymer fibres extend, they deform the erythrocytes and interfere with their flexibility and rheological properties (that is, how they flow), which eventually results in vaso-occlusion[50]. This impaired blood flow rheology is worsened by erythrocyte aggregation, especially in patients with SCD and high haematocrit (the percentage of blood volume composed of erythrocytes)[50]. Repeated episodes of HbS polymerization and erythrocyte sickling in low pO_2 and unsickling in high pO_2 can lead to severe alterations in the membrane structure and function (see below) and abnormal calcium compartmentalization. Membrane deformation and erythrocyte dehydration eventually results in the formation of an irreversibly sickled cell, a sickle erythrocyte that no longer can revert to its natural shape[51-54].

[H3] Altered erythrocyte membrane biology. HbS polymerization directly or indirectly alters the typical lipid bilayer and proteins of the erythrocyte membrane, which leads to reduced cellular hydration, increases haemolysis, abnormal interactions with other blood cells and contributes to early erythrocyte apoptosis[54-57] (Figure 4). Several membrane ion channels are dysfunctional, including the K-Cl cotransporter 1 (KCC1, also known as solute carrier family 12 member 4), KCC3 (also known as solute carrier family 12 member 6) and KCC4 (also known as solute carrier family 12 member 7), the Gardos channel (encoded by *KCNN4*) and P_{sickle}, the polymerization induced membrane permeability, most likely mediated by the piezo-type mechano-sensitive ion channel component 1 (PIEZO1), resulting in reduced cellular hydration[49]. In a subpopulation of sickle erythrocytes, phosphatidylserine (which is usually confined to the inner layer of the membrane) is exposed on the erythrocyte surface. Circulating phosphatidylserine-exposing erythrocytes have a role in many important pathophysiological events, including increased haemolysis; endothelial activation; interaction between erythrocytes, white blood cells and platelets; and activation of coagulation pathways[58,59]. HbS polymers and HbS oxidation (see below) also affect membrane proteins that also have structural functions, especially the band 3 anion transport protein, and these changes lead to membrane microvesiculation and the release of erythrocytes microparticles[60,61]. These sub-micron, unilamellar vesicles are shed from the plasma membrane under cellular stress to the membrane and cytoskeleton. They are derived in large numbers in SCD from erythrocytes[62], but also from platelets, monocytes and endothelial cells. Microvesicles possess cell surface markers, cytoplasmic proteins and micro RNAs derived from their cell of origin and can affect coagulation, adhesion, inflammation and endothelial function[63,64]. By contrast, exosomes originate from the endosomal system[65], and have been less studied in SCD.

[H2] HAEMOLYSIS

Sickle erythrocytes are highly unstable, with a lifespan that is reduced by ≥75%[64,66]. Haemolysis is thought to occur principally via extravascular phagocytosis by macrophages, but a substantial fraction (roughly one-third) occurs through intravascular haemolysis (Figure 4)[67]. It has been hypothesized that the rate of intravascular haemolysis in SCD is insufficient to produce a clinical phenotype, including

pulmonary hypertension[68], the most serious consequence of intravascular haemolysis. However, the epidemiological, biochemical, genetic and physiological data supporting a link between intravascular haemolysis and vasculopathy continue to expand[69].

[H3] Oxidative stress. Haemolysis is both a cause and effect of oxidative stress. The substantial levels of oxidative stress in sickle erythrocytes enhance HbS autoxidation, which could contribute to the damage of the cell membrane, premature erythrocyte aging and haemolysis[64]. In addition to the accelerated autoxidation of HbS, oxygen radicals result from increased expression of oxidases, especially xanthine dehydrogenase/oxidase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[70,71], extracellular heme and Hb in plasma and probably also from recurrent ischemia-reperfusion of tissues. Cytoskeletal proteins and membrane lipids become oxidized and this chronic severe oxidative stress in sickle erythrocytes depletes the levels of catalytic antioxidants[64] such as superoxide dismutase, peroxiredoxin-2 and peroxiredoxin-4 (Ref[45,72]). This issue is worsened by depletion of the endogenous reductant glutathione[45,73]; impaired antioxidant capacity probably contributes to haemolysis.

[H3] Free plasma Hb and heme. Extracellular Hb (in plasma or in microparticles[63,64]) and heme in plasma promote severe oxidative stress, especially to blood vessels and blood cells[64]. Continuous autoxidation of extracellular Hb produces superoxide which dismutates into hydrogen peroxide (H_2O_2), a source for additional potent oxidative species, including the ferryl ion, which promotes vasoconstriction[64]. Extracellular Hb scavenges nitric oxide (NO, which is generated by NO synthase (NOS) in endothelial cells and promotes vasodilation) ~1,000-fold more rapidly than cytoplasmic Hb, thereby decreasing NO bioavailability[74]. This results in vascular dysfunction, indicated by impaired vasodilatory response to NO donors, activation of endothelial cells (producing cell surface expression of endothelial adhesion molecules, and detected by elaboration of soluble ectodomains of the adhesion molecules into plasma) and haemostatic activation of platelets, indicated by cell surface expression of P-selectin (which mediates the interaction between activated platelets and leukocytes) and activated integrin α-IIb/β-3[69]. Markers of haemolytic severity (such as low haemoglobin or high serum lactate dehydrogenase) predict clinical risk of developing vascular disease complications (see below).

[H3] Disruption of arginine metabolism. Intravascular haemolysis releases two factors that interfere with NOS activity. The enzyme arginase-1 competes with NOS for L-arginine, the substrate required for NO production by NOS[75]. Arginase-1 converts L-arginine into ornithine, which fuels the synthesis of polyamines, which in turn facilitate cell proliferation[76], potentially of vascular cells, probably promoting vascular remodelling. Asymmetric dimethylarginine (ADMA) is an endogenous NOS inhibitor and a proteolytic product of proteins methylated on arginine; ADMA is abundant in erythrocytes and also released during haemolysis[77]. Both ADMA and depletion of L-arginine by arginase-1 could contribute to uncoupling of NOS, which then produces reactive oxygen species (ROS) instead of NO [78,79].

[H3] Plasma lipids. Patients with SCA often have a form of dyslipidaemia that is associated with vasculopathy: triglyceride levels are high and correlate with haemolytic severity[80]. Although total

cholesterol levels are generally low in patients with SCA, the levels of apolipoprotein A-I (which promotes hepatic cholesterol catabolism and promotes NOS activity) are particularly low, especially during vaso-occlusive pain crisis and in association with markers of pulmonary hypertension and endothelial dysfunction[81]. Genetic variants of apolipoprotein L1 have been associated with renal disease in SCA[82].

[H2] INNATE IMMUNE SYSTEM ACTIVATION

Plasma heme and Hb act as danger-associated molecular patterns (DAMPs) to activate the innate immune system and heighten the adhesiveness of circulating blood cells to each other and to the endothelium, thereby triggering vaso-occlusion[69] (**Figure 4**). Heme activates neutrophils to release DNA as neutrophil extracellular traps (NETs) that increase platelet activation and thrombosis, promotes pulmonary vaso-occlusion[83] and release of placenta growth factor from erythroblasts (nucleated precursors of erythrocytes). Placenta growth factor is a ligand for vascular endothelial growth factor receptor 1 on endothelial cells and macrophages, promoting release of endothelin-1, which contributes to pulmonary hypertension[84]. The toll-like receptor-4 (TLR4) is highly expressed in immune cells in SCD, and tissue damage and platelet activation release high mobility group protein B1 (HMGB1), a high-affinity TLR4 ligand. TLR4 also binds lipopolysaccharide (LPS) derived from gram-negative bacteria, which could explain why infections promote vaso-occlusive crises in patients with SCA. Ligands of TLR4 activate monocytes and macrophages to release inflammatory cytokines, which promote an inflammatory state and activate adhesiveness of neutrophils, platelets and endothelial cells. Finally, increased intracellular iron from turnover of haemolyzed and transfused erythrocytes is associated with markedly increased expression in peripheral blood mononuclear cells of several components of the inflammasome pathway[85].

[H2] CELL ADHESION AND VASO-OCCLUSION

[H3] Endothelium activation. Vaso-occlusion in SCA is a complex phenomenon in which interactions between erythrocytes and endothelial cells, leukocytes and platelets play a central part (**Figure 4**). Endothelial cells are probably activated by direct contact of sickle erythrocytes, free heme and Hb and hypoxia-induced ROS[86]. Reduced NO bioavailability could induce the expression of adhesion molecules and production of endothelin-1 (a vasoconstrictor). The increased expression of endothelial adhesion molecules such as vascular cell adhesion protein 1 (VCAM-1)[87,88], intercellular adhesion molecule 1 (ICAM-1)[89], P-selectin, E-selectin, leukocyte surface antigen CD47, integrins α-V/β-3, exposed heparin sulphate proteoglycans and phosphatidylserine are responsible for erythrocyte and leukocyte adhesion[88]. Activated endothelial cells also produce inflammatory mediators, such as IL-1β, IL-6 and tumour necrosis factor (TNF), which lead to a chronic inflammatory state.

[H3] Erythrocytes. Sickle erythrocytes are more adhesive to endothelial cells than normal erythrocytes [86,90]. Many adhesion molecules (the most important include integrins α-4/β-1 (also known as very late antigen 4 (VLA-4), which is reticulocyte-specific), platelet glycoprotein 4 (also known as CD36) and basal cell adhesion molecule (BCAM)) are overexpressed by sickle red blood cells and mediate the adhesion to the endothelium[91]. Interestingly, reticulocytes and deformable erythrocytes (that is, erythrocytes that

have not become permanently sickled) are substantially more adhesive than the irreversible and dense sickle erythrocytes[92].

[H3] Leukocytes. High baseline leukocyte numbers are associated with increased morbidity and mortality in SCA[93,94]. Many studies in mouse models of SCA indicate that neutrophils have an important role in vaso-occlusion; neutrophils adhere to the endothelium and sickle erythrocytes could bind to these cells, thereby reducing blood flow and promoting vaso-occlusion[95]. Indeed, neutrophils are in an activated state in SCA and have increased expression of integrins α-M/β-2 (also known as macrophage-1 antigen) with enhanced adhesion to endothelial and sub-endothelial proteins (such as fibronectin)[96]. Selectins produced by activated endothelium have an important role in the initial binding of neutrophils to the vascular wall[95].

[H3] Platelets. Platelets play an important part in the pathophysiology of SCA and are in an activated state[95],with high levels of P-selectin and activated integrins α-IIb/β-3. Moreover, several biological markers of activated platelets are increased in SCA, for example, platelet microparticles[63], thrombospondin[92], platelet factor 4 (also known as C-X-C motif chemokine 4 (CXCL4)) and β-thromboglobulin. Platelets are found in circulating heterocellular aggregates of neutrophils and red blood cells (mainly reticulocytes) in the blood from patients with SCA, and their adhesion to these aggregates is mediated in part through P-selectin[97]. These data strongly suggest that platelets have a role in the formation of these aggregates. Platelets could also act as accessory cells of the innate immune system, by releasing cytokines[98].

[H1] Diagnosis, screening and prevention

[H2] Diagnostic opportunities

The goals and methods of diagnosis of SCD vary with the age of the person. In general, there are 4 overlapping testing periods: preconception, prenatal, neonatal and post neonatal. The preconception testing is designed to identify asymptomatic potential parents whose offspring would be at risk for SCD. Laboratory techniques used for preconception testing are routine basic methods of protein chemistry that enable to separate hemoglobin species according to their protein structure, including hemoglobin electrophoresis, high-performance liquid chromatography and isoelectric focusing[99]. Prenatal diagnosis is a relatively safe but invasive procedure and is offered during early pregnancy to couples who tested positive at preconception screening. It requires fetal DNA samples obtained from chorionic villus analysis performed at 9 weeks gestation[99]. Non-invasive prenatal diagnosis techniques are being developed but still investigational. These new techniques can detect fetal DNA in maternal circulation as early as by 4 weeks of gestation. Some couples who test positive at preconception screening might opt for in vitro fertilization with pre-implementation genetic diagnosis, if available, to genetically identify at risk embryos before embryo transfer occurs[100].

[H3] Newborn screening

Newborn screening for SCD is performed at birth before symptoms occur, utilizing haemoglobin protein analysis methodologies. Two types of newborn screening programmes have been used, selective screening of infants of high risk parents (targeted screening) and universal screening. Universal screening is generally more cost effective, identifies more newborn babies with disease and prevents more deaths [17,101]. In areas without newborn screening programmes, the initial diagnosis of SCD occurs at approximately 21 months of age[102]. In many cases, the initial presentation is a fatal infection or acute splenic sequestration crisis[102]. Early diagnosis accompanied by penicillin prophylaxis and family education reduces the mortality in the first five years of life from 25% to <3%[102,103]. Similar positive results are found in low-income countries[104,105].

[H3] Post neonatal testing

The requirement of post neonatal testing for SCD is influenced by several factors that affect the population's knowledge of their SCD status. These factors include regional success of neonatal screening, immigration of at risk patients not previously tested, and access to neonatal results in older patients. [106]. HbAS is a benign condition and not a disease, but is also a risk factor for uncommon serious complications[106]. Thus, knowledge of HbAS status is important in the prevention of rare serious complications as well as family planning.

HbAS can also be detected by newborn screening programmes, but HbAS detection is not the primary objective and many programmes do not provide this information or offer associated counselling. Individuals who wish to have children should be screened to discover heterozygous genotypes that could be important in genetic counselling. HbAS screening enables informed decisions concerning preconception counseling and prenatal diagnosis.

Routine fitness training does not increase the risk of mortality for individuals with HbAS. However, there is a concern of increased risk for rhabdomyolysis (rapid destruction of skeletal muscle) and sudden death during intense prolonged physical activity that can be mitigated by proper training[107]. These observations have resulted in some regions in voluntary or mandatory screening of athletes for HbAS[106] . There are rare and specific complications of HbAS that should prompt HbAS testing. These include hematuria (blood in the urine), hyphema (blood inside the eye's anterior chamber), and renal medullary carcinoma, a rare malignancy. HbAS could be a risk factor for chronic kidney disease and pulmonary embolism [108]

[H2] NEWBORN BABIES SCREENING

[H3] Screening in Europe

Newborn babies screening for SCD in the United Kingdom became universal in 2006 (Ref[109]); the primary aim of the programme is to diagnose SCD, but if a baby has HbAS the parents are provided with specific informational materials. In France, screening for SCD has been in place since 2000, but is restricted to newborn babies whose parents both originate from SCD-endemic regions[110]. In Spain, universal screening has been recommended for regions with high annual birth rate and SCD prevalence (Catalonia

and Madrid, for example), whereas targeted screening is recommended for regions with low annual birth rate and SCD prevalence.[111] Screening programmes are also present in Italy[112] and Germany[113].

[H3] Screening in the USA. In the United States, state-wide newborn babies screening originated in New York state in 1975 (**Box 1**) and by 2007 all states had universal screening programmes[21]. In the United States, high-performance liquid chromatography (HPLC) and isoelectric focusing are the predominant screening methods[21,99]. Confirmation of the diagnosis by DNA analyses to detect haemoglobin variants is commonly used, but not standardized between states. A major gap in these programmes is the lack of follow-up and variability of state-wide education programmes[114]. The identification of substantial clinical morbidity occasionally associated with individuals with HbAS has not yet resulted in routine counselling and genetic testing of family members of newborn babies who have HbAS[106].

[H3] Screening in India. The population of India consists of >2,000 different ethnic groups, most of which have practiced endogamy (the custom of marrying only within the limits of the local community) over centuries. Thus, although the β^S allele has been detected in many ethnic groups, its prevalence has been enriched in some. The at-risk population consists of several hundreds of millions of individuals, predominantly belonging to historically disadvantaged groups [115]. Screening efforts have focused on groups with high prevalence of β^S allele and areas with large numbers of these at-risk populations. Screening typically consists of haemoglobin solubility test (a screening test that does not distinguish HbS trait **[Au: "HbAS"?]** from disease) at the point of care, with further testing of initial positive samples **[Au:OK?]** by HPLC analysis at a reference centre. Screening programmes also includes education, testing and genetic counselling. In many hospitals, such services are also offered to relatives of patients diagnosed with SCD, as well as in the prenatal setting to mothers either previously diagnosed with HbAS or belonging to an at-risk ethnic group. Pilot projects of newborn babies screening for SCD have been implemented in the states of Gujarat, Maharashtra and Chattisgarh[104,105,116-119], which resulted in detailed data on the prevalence of HbAS in various populations, with ranges of 2-40%. There is considerable regional variation in the implementation of follow-up approaches such as comprehensive care, penicillin prophylaxis and immunization against pneumococcus.

[H3] Screening in Africa.

No country in sub-Saharan Africa has implemented a universal newborn babies screening programme for any disease.[120] However, a few countries in sub-Saharan Africa have developed pilot newborn babies screening programmes on SCD. Among these, Ghana's National New-born Screening Programme for SCD, launched in 2010 following a 15-year pilot study, is the most developed[121] (Box 2). Other countries in Africa where small-scale or pilot newborn babies screening for SCD has been conducted or is ongoing include Angola[122], Benin[123], Burkina Faso[124], Burundi[125], Congo (DR)[126], Nigeria[127], Rwanda[125], Senegal[128], Tanzania[129] and Uganda[130]. Screening followed by penicillin prophylaxis can reduce early mortality from pneumococcal bacteremia[102,103]. Nevertheless, current and future numbers of patients with SCA or HbAS make the scalability of the interventions implemented in high-income, low-burden countries (such as universal newborn babies screening programmes) in low-resource settings challenging. There is no mandatory or large scale preconception screening programme for adults who wish to have children in any African country. However, several churches require couples to be screened for SCD-related conditions as a pre-requisite for marriage approval. Such screening often involves inexpensive but inconclusive "sickling" and solubility tests, which cannot identify individuals with the β^C allele or β-thalassaemia, conditions that, although not characterized by the presence of HbS, are of genetic counselling relevance. There are very few much-needed certified genetic counsellors to support the screening programmes. The Sickle Cell Foundation of Ghana launched the first Sickle Cell Genetic Counsellor Training and Certification Programme in June 2015 (**Box 2**).

[H2] PHENOTYPES IN SCD

There is great phenotypic variability among patients with SCD. Some variability shows a specific geographical distribution and is associated with known or suspected genetic variants[131]. However, some complications cluster together epidemiologically in subphenotypes, at times united by a common biomarker that suggests a mechanism, such as particularly low haemoglobin level with high reticulocyte count or high serum LDH level, implying more-intense haemolysis. These phenotypes are not mutually exclusive, exist often as a spectrum, can overlap, are probably due to independent genetic modifiers of the underlying mechanisms and might change with aging.

[H3] Vaso-occlusive subphenotype. This SCA subphenotype is characterized by higher haematocrit than other individuals with SCA**,** which promotes high blood viscosity. Patients with this phenotype are predisposed to frequent vaso-occlusive pain crisis, acute chest syndrome (that is, a vaso-occlusive crisis of the pulmonary vasculature) and osteonecrosis. Co-inheritance of α-thalassaemia reduces haemolysis, but promotes higher haematocrit (by reducing intracellular concentration of HbS, which slows HbS polymerization and haemolysis)[132] .

[H3] Haemolysis and vasculopathy subphenotype. This phenotype is characterized by lower haematocrit than that of individuals with the vaso-occlusive subphenotype accompanied by higher levels of serum lactate dehydrogenase and bilirubin, which indicate more-severe haemolytic anaemia. Patients in this group are at risk for ischaemic stroke, pulmonary hypertension, leg ulceration, gall stones, priapism

(persistent and painful erection) and possibly nephropathy[133]. Decreased NO bioavailability, heme exposure and heme turnover are associated with these vasculopathic complications. The severe anaemia also promotes high cardiac output as a compensatory mechanism, and this excessive blood flow has been suggested to promote vasculopathy in the kidney and potentially other organs.

[H3] High HbF subphenotype. Persistent expression of HbF in the range of 10-25% of total haemoglobin owing to genetic variants generally reduces the clinical severity of SCA[3,134]. However, not all patients with the common, uneven cellular distribution of HbF (heterocellular distribution) have a mild phenotype. Expression levels of 25-50% of HbF in every erythrocyte (pancellular distribution) lead to nearly complete amelioration of SCA, with rare clinical symptoms and no anemia[135], a finding that could prompt the development of drugs that can induce 'globin switching' (that is, the preferential expression of *HBG1* and *HBG2*).

[H3] Pain subphenotypes. Patients with pain-sensitive or pain-protective phenotypes experience pain differently, potentially owing to altered neurophysiology of pain sensation pathways. One example of a genetic modifier of pain is *GCH1*, which is associated with pain sensitivity in healthy individuals and a variant of *GCH1* is associated with frequency of severe pain in SCA[136]. Quantitative sensory testing of pain sensitivity is being used to functionally characterize these phenotypes in SCA[137].

[H1] Management

SCD is a complex, multisystem condition characterized by acute and chronic complications (**Figure 5**). Advances in general medical care, early diagnosis and comprehensive treatment have led to substantial improvements in the life expectancy of patients with SCA in high-income countries[8,9] as almost all patients survive beyond 18 years of age[138]. However, even with the best of care, life expectancy is still reduced by ~30 years, second, routine and emergency care for patients with SCD have great financial costs, the quality of life often deteriorates during adulthood and the social and psychological effects of SCD on patients and their families remain underappreciated[139]. Furthermore, most of these advances have not reached low-income countries[140].

[H2] THERAPIES

Three therapies modify the disease course of SCA: hydroxycarbamide, erythrocyte transfusion, and haematopoietic stem cell transplantation[141].

[H3] Hydroxycarbamide. Hydroxycarbamide (alternatively known in some countries as hydroxyurea), a ribonucleotide reductase inhibitor, has multiple physiologic effects, including increasing HbF expression (in most patients with SCA [142]) and decreasing leukocyte count. It was approved by the FDA in 1998 and by the EMA in 2007 for the treatment of SCD. The drug significantly reduces the incidence of SCA vaso-occlusive crisis events, hospitalizations and mortality in high-income countries (with studies ongoing in low-resource countries) with an excellent safety profile[143], although some patients do not have a beneficial response, usually because of limitations of adherence to treatment[144], but possibly sometimes for pharmacogenomic reasons[145]. Hydroxycarbamide is underutilized because of healthcare

infrastructure deficiencies in both low-resource and high-resource countries and disproportionate perceptions of carcinogenicity, teratogenicity and reduced fertility --which have not been problems so far in follow-up studies[142,148,149], although utilization is increasing. Snapshots from various cohorts over the years show that in high-resource countries, at specialized SCD clinics up to 63% of SCA patients may be on hydroxycarbamide[146], but the percentage is near zero in most African countries[147]. Because of very favourable clinical trial results in infants and toddlers[150], hydroxycarbamide is prescribed with increasing frequency to children with SCA, up to 45% in multinational SCD centers[151]. Although there is still limited evidence on whether hydroxycarbamide improves survival and prevents SCD complications in low-income countries[152], various studies, including the Realizing Effectiveness Across Continents with Hydroxyurea (REACH) trial, are currently underway and should address knowledge gaps about treatment options for SCA in sub-Saharan Africa[147].

[H3] Erythrocyte transfusion. This therapy improves microvascular flow by decreasing circulating sickle erythrocytes and is associated with decreased endothelial injury and inflammatory damage[153,154]. Chronic transfusion therapy, prescribed in high-resource countries primarily to the roughly 10% of SCA patients at high risk for stroke, can ameliorate and prevent stroke and vaso-occlusive crisis [155]; however, several potential adverse effects, including iron overload, alloimmunization (an immune response to foreign antigens that are present in the donor's blood) and haemolytic transfusion reactions, limit its potential benefits. The availability of oral iron chelating drugs since 2005 has reduced the adverse effects of iron overload. In countries with limited testing of blood products for infectious agents, there are substantial risks of transmission of blood-borne infections, such as hepatitis B, hepatitis C, HIV, West Nile Virus infection and others. Transfusion protocols with extended erythrocyte matching that include the erythrocyte antigens Kell, C, E and Jkb and iron chelation therapy guidelines improve the safety of this therapy[155]. Systematic genotyping of blood groups for the patient has been proposed to reduce alloimmunization[156].

[H3] Haematopoietic stem cell transplantation. Haematopoietic stem cell transplantation in SCA is curative and should be considered in symptomatic patients with an HLA-matched family donor. Worldwide, it is estimated that nearly 2,000 patients with SCA have undergone allogeneic haematopoietic stem cell transplantation; the survival exceeds 90% in US and European studies[157,158]. In pooled registry data, the average rate of both acute and chronic graft versus host disease has been 14%, and is generally lower with newer approaches[157] , and the rate of graft failure has been 2%[158]. Early results with experimental reduced-intensity conditioning regimens (the pre-transplantation chemotherapy to ablate or suppress the recipient's bone marrow) are very encouraging[159]. However, most patients do not have an HLA-matched related donor. Experimental use of expanded donor pools (haploidentical donors (who share 50% of the HLA antigens with the recipient) and unrelated HLA-matched donors) can increase the probability of cure, but also increase the rates of graft rejection and mortality, rates that seem to improve with ongoing research[160]. Although haematopoietic stem cell transplantation from the bone marrow of a healthy HLA-matched donor can cure SCA, this therapy is limited by the paucity of suitable donors and is only available in high-income countries[161].

[H2] MANAGEMENT OF ACUTE COMPLICATIONS

The principles of management of acute complications in SCA (**Figure 5**) include the need for early diagnosis, consideration of other non-SCD-related causes and rapid initiation of treatment. The use of standardized protocols for common complications improves outcome.

[H3] Acute pain. Acute pain events usually affecting the extremities, chest and back are the most common cause of hospitalization for patients with SCA. However, the majority of such events are managed at home with NSAIDs or non-prescription oral opioid analgesics without the involvement of the health provider. The pathophysiology and natural history of acute pain events are complex and treatment is suboptimal[162]. Individual personalized protocols for outpatient and inpatient pain management improve quality of life and decrease hospital admissions[163-165]. The treatment is guided by the severity of pain, which is generally self-reported using pain severity scales. When home management with oral analgesics, hydration and rest is ineffective, rapid triage with timely administration of opioids is recommended. Initial treatment in a day unit compared with an emergency room drastically decreases hospitalization[166]. Initiation of treatment for emergency room patients with SCD is often markedly delayed, with patients with SCD waiting 25 to 50% longer than patients without SCD with similar pain acuity [167]. In some programmes, innovative emergency room treatment protocols for patients with SCD using standardized time-specific dosing protocols and intranasal fentanyl have substantially reduced time to treatment; similar approaches should be adopted universally[163,164]. Once hospitalized, a standardized protocol using patient-controlled analgesia devices is indicated. These intravenous infusion pumps allow for patient self-medication and in general result in improved analgesic control and less analgesic use[168]. Incentive spirometry, a simple device that prevents atelectasis (the complete or partial collapse of a lung), with close monitoring of the patient's level of sedation, hydration, and oxygenation improves outcomes. Although intensive analgesia is important to effective medical management of pain in SCD, in some countries opioids are unavailable owing to resource limitations or are not prescribed or assumed owing to stigma[169]. Vaso-occlusive crisis can sometimes result in sudden unexpected death[3,170]. The precise aetiology of sudden death in such cases is unclear, although autopsy often shows histopathological evidence of pulmonary arterial hypertension[170].

[H3] Acute chest syndrome. Acute chest syndrome is the second most frequent reason for hospitalization and a leading cause of death in patients with SCD — it is often linked to and following an acute pain event[171]. The severity of acute chest syndrome increases with age. In adults, >10% of cases are fatal or complicated by neurologic events and multi-organ failure[172]. The initial pulmonary injury is multifactorial, including infection, pulmonary fat embolism, pulmonary infarction and pulmonary embolism[173]. The presence of underlying, often undetected bronchoreactive lung disease can increase the frequency and severity of acute chest syndrome events[174]. Early chest x-ray imaging tests and oxygen monitoring of patients with any pulmonary symptoms is necessary. Hospitalization with broad-spectrum antibiotics, bronchodilators, oxygen supplementation and red cell transfusions are often indicated[175]. Exchange transfusions (in which the patient's blood is replaced by donor blood) and steroids, which decrease acute inflammation, could modify a severe or rapidly deteriorating event[176]. Exchange transfusion is the most effective method to lower the level of HbS below 30% of the total Hb without raising the total Hb level above 10 gm/dL[177]. However, delayed transfusion reactions can complicate transfusion therapy and present as a hyper-haemolytic episode in which the transfused cells

and the patient's own red cells are destroyed[178].Steroids often provide benefit but are associated with ~25% risk of mild or severe complications (in particular, there is a high rate of recurrence of acute chest syndrome once the steroids are stopped), so their use is usually limited to life-threatening acute chest syndrome events[179].

[H3] Acute stroke. An acute stroke, including ischaemic and haemorrhagic events, is a medical emergency. Children with SCA have a 300-fold higher risk rate of acute stroke than other children without SCD, and by 45 years of age one in four adults with SCA has had a stroke[180]. In the United States, 25% of patients with SCA develop an overt stroke, and another 35% have non-focal CNS injury[180-182]. Ischaemic stroke is usually caused by occlusion of a large cerebral artery and can occur as complication of a pulmonary or other sickle event or independently and manifest with transient ischaemic attack, sudden weakness or loss of consciousness. Prompt evaluation (including MRI of patients with more-subtle presentations) is indicated. Rapid exchange transfusion is the standard treatment. In addition, exchange transfusion decreases secondary stroke recurrence[183]. The importance of subsequent monthly chronic transfusion to prevent secondary stroke has been re-affirmed by the Stroke With Transfusions Changing to Hydroxyurea (SWITCH) study[184].

Intracranial haemorrhage or haemorrhagic stroke account for 3–30% of acute neurological events, and have a 25–50% acute mortality rate[183]. Clinically, these patients present with severe headache or loss of consciousness without hemiparesis. Imaging with angiography could reveal a surgically treatable aneurysm. Patients with moyamoya vasculopathy, which is a prominent collateral circulation around occluded arteries of the circle of Willis that is frequent in individuals with SCD, are at high risk for intracranial bleeding. When electively detected, indirect revascularization using encephaloduroarteriosynangiosis (a surgical procedure that implants the superficial temporal artery to the brain surface increasing blood flow to the ischemic area) is often considered to decrease bleeding risk and improve oxygenation[185,186].

[H3] Acute anaemic events. Over half the patients at some point in their life will experience an acute anaemic event, which can be fatal. The most common types of anaemic events are splenic sequestration crisis, aplastic crisis (temporary absence of erythropoiesis), and hyper haemolytic crisis are the most common causes. Acute splenic sequestration crisis is characterized by rapid swelling of the spleen and hypovolemia with a sudden fall in Hb levels. As many as 30% of young children experience acute sequestration events, which are a leading cause of infant mortality. Early detection is crucial, and usually transfusion followed by elective splenectomy are required[187]. Nonsurgical supportive care can be successful, and when necessary, transfusion with extended red cell antigen matched erythrocyte units and selective use of immunosuppressive therapy are indicated.

[H3] Cholelithiasis. Cholelithiasis (gallstones) results from the chronic accelerated rate of erythrocytes destruction in patients with SCD. The heme is metabolized to bilirubin, which in the bile can form insoluble calcium bilirubinate, which in turn precipitates as a pigment and forms gallstones. Of note, a variant of *UGT1A1* (which encodes a protein involved in bilirubin processing) increases bilirubin

metabolism and, therefore, the formation of gallstones in patients with SCD[188]. By the time of adulthood (**Figure 6**), 20% of patients have acute complications from gallstones, which can promote cholecystitis (inflammation of the gall bladder) and often necessitates cholecystectomy (surgical removal of the gall bladder)[189]. By contrast, patients with SCD who also inherit α-thalassemia have reduced haemolysis, bilirubin production and gallstone formation[188].

[H2] LONG-TERM MANAGEMENT

Improved management of acute complications is associated with a longer survival. As patient with SCD age, chronic problems resulting from cumulative organ injury can lead to severe morbidity (**Figures 5 and 6**)[190]. Chronic pain is common (the Pain in Sickle Cell Epidemiology Study (PiSCES) found that adults with SCD have pain in 55% of days[191] and pain, in general, is a poorly managed complication of SCD[192]. Patients with SCD and recurrent pain have altered brain network connectivity, which affects their response to treatment[193]. Chronic pain requires a multidisciplinary team familiar with neuropathic pain tolerance, withdrawal symptoms and hyper analgesia syndrome[192]. Hydroxycarbamide, selective use of chronic transfusions in severe patients and long acting opioids are useful components of a multidisciplinary pain management approach.

Avascular necrosis of the hip is a common cause of chronic pain that eventually develops in many patients[194]; in >20% of hospitalizations, symptoms are related to avascular necrosis. Although core decompression (in which a small core of bone is removed from the damaged area, lowering the bone marrow pressure and stimulating healthy bone regrowth), physiatry (rehabilitation) therapy and analgesics temporarily are helpful, total hip replacement is often required.

Chronic kidney disease is relatively common in older patients and thought to have a poor prognosis in these patients compared with patients without SCD [195]. This worse outcome could in part be due to delayed access to dialysis and renal transplant for patients with SCD, as they might not be considered as good candidates for these therapies. Of note, patients with SCD who receive a timely renal transplantation have an outcome comparable with patients without SCD who receive a transplant[196,197].

Although screening for brain injury with annual transcranial Doppler and/or MRI imaging and chronic transfusion therapy for high-risk patients decrease the frequency and severity of stroke complications, patients continue to have progressive neurocognitive injury and require close observation and long term therapy[181]. In addition, implementation of multidisciplinary plans for management of other common chronic complications of SCD (for example, cardiopulmonary dysfunction, priapism and leg ulcers) improve the quality of life of these patients as they age[198,199].

[H2] PREVENTION OF COMPLICATIONS

Preventative strategies have changed the long-term outcome in SCD more than any other approach. Prevention of life-threatening infections and stroke has drastically reduced childhood mortality in SCD; generalized screening of patients for risk factors and early evidence of disease enables the implementation of treatment that can reduce morbidity. Screening for pulmonary, renal and systemic hypertension, retinopathy, and damage to other organs are indicated[200]. Detailed generalized screening recommendations for SCD are available[201].

[H3] Prevention of infection. Until 1990s, in the United States, up to 30% of young children with SCA died from infections, predominantly due to encapsulated bacteria[103], caused by a common childhood deficiency of immune response to polysaccharide antigens[202], exacerbated in SCA by impaired clearance of bloodborne bacteria caused by functional asplenia[103]. The introduction of prophylactic penicillin treatment decreased the incidence of pneumococcal bacteraemia associated with impaired splenic function by 85%[103]. Prophylactic penicillin has remained safe and beneficial in patients through at least five years of age. The universal use of pneumococcal and other standard vaccinations has further lowered infectious disease mortality. The first conjugated pneumococcal vaccine decreased the rate of pneumococcal bacteraemia in children under 3 years of age by 93.4% and added protection to the large cohort of patients who have suboptimal compliance with prophylactic penicillin therapy[203]. Long-term penicillin prophylaxis has raised concerns about the development of penicillin-resistant pneumococcal colonization and disease[204], especially in low-income countries, although the benefit to risk ratio of prophylaxis is still high. The pneumococcal conjugate vaccine PCV13 and pneumococcal polysaccharide vaccine PPSV23[205] can prevent infection by most – but not all – serotypes.

[H3] Prevention of central nervous system (CNS) injury. Cerebral vascular injury and neuro-ischaemic damage are a leading cause of death and morbidity in children and adults with SCA. The complications of these events are largely irreversible and mandate universal prevention and screening policies. Transcranial Doppler (TCD) screening to detect increased vascular velocity can contribute to identify children at high risk for stroke, which can be largely prevented by initiating transfusion therapy[206]. The landmark Stroke Prevention Trial in Sickle Cell Anemia (STOP) demonstrated that neurologically normal children with elevated TCD measurements (vascular velocity > 200 cm/sec) are at high risk for stroke, and chronic monthly transfusions reduced the rate of strokes from ~11% to 1%[206]. These findings suggest that all children with SCA should be screened annually with TCD. The STOP II study found that discontinuing these preventive transfusions was not safe and transfusion therapy for an indefinite period of time might be necessary[207].

Nevertheless, chronic transfusion therapy for primary stroke prevention is associated with substantial complications and not available in many low-income countries. Hydroxycarbamide therapy has been associated with decreased TCD vascular velocity[208].The TCD with Transfusions Changing to Hydroxyurea (TWiTCH) trial determined that hydroxycarbamide therapy at maximum dosing was non-inferior to blood transfusions for primary stroke prevention in children with non-severe vasculopathy on MRI findings and who had been receiving transfusions for ≥1 year[209]. The Stroke Prevention Study in Nigeria (SPIN) provided pilot evidence that TCD screening followed by fixed-dose hydroxycarbamide therapy is feasible and has the potential to prevent strokes in low-resource areas[210]. Global TCD screening of all children with SCA is a major public health priority.

TCD screening does not detect silent infarction involving small vessel disease, which is a major cause of neurocognitive impairment in SCD. The Silent Cerebral Infarct Transfusion Multi-Center Clinical Trial (SIT) screened with MRI children who had normal TCD measurements and no neurological symptoms[211]. Children with small non-focal cerebral infarctions (detected by MRI) were randomly assigned to receive transfusion or observation. Patients in the transfusion group had a 59% relative risk reduction for stroke. Whether all children should be screened with MRI remains debated. However, all patients with soft (subtle) neurological signs or neurocognitive changes (such as sudden unexplained decline in school or work performance) should undergo MRI screening, and those with silent infarction should be offered transfusion therapy. Neurocognitive testing, where available, is a useful tool in identifying patients who have non-focal ischaemic cerebral injury, which can progress with age and is common in adults with no neurological symptoms[181].

[H3]Prevention of pulmonary complications.

Pulmonary disease is a leading cause of morbidity and mortality in patients with SCD[3,190,212]. Asthma is an independent predictor of mortality in this population[213,214]. Unrecognized bronchoreactive lung disease is common in paediatric patients and increases the severity and frequency of acute chest syndrome events. Many adults have undetected, restrictive chronic lung disease, which is a risk factor of pulmonary failure and myocardial injury[215]. Incorporating respiratory symptom questionnaires and routine spirometry into outpatient management is indicated. Pulmonary hypertension or an elevation in the tricuspid regurgitant jet velocity (TRV), which is a marker of pulmonary hypertension, are also independent predictors of mortality. Patients with TRV ≥3cm/sec have a 10-fold increased mortality compared with patients with normal TRV[199]. The American Thoracic Society recommends that all adults with SCA undergo serial echocardiography every one to three years to detect pulmonary hypertension[216].

[H3] Prevention of renal complications

One-third of patients with SCA develop chronic kidney disease and up to 18% of patients with SCA require dialysis or renal transplantation[217]. Proteinuria is strongly associated with progressive disease; serial urinary screening for proteinuria accompanied with treatment with angiotensin-converting enzyme inhibitors (which correct the proteinuria) could lower the risk of chronic kidney disease[200]. Mild systemic hypertension (120-139/80-90 mmHg) increases the risk of stroke, pulmonary hypertension, nephropathy, mortality and hospitalization in SCD [218,219], and early diagnosis and treatment is beneficial[219,220]. Asymptomatic proliferative retinopathy can occur in up to 43% of patients with HbSC disease and 14% of patients with SCA[221]; if untreated, it results in loss of visual acuity[222].

[H2] CO-MORBIDITIES

Patients with SCD are subject to other unrelated diseases that can modify each patient's clinical course. Very common (in at least one-third of patients) co-morbidities identified using screening questionnaires are depression and anxiety[223,224]. Depression and anxiety are associated with greater sensitivity to

pain[225], and greater health care utilization[226]. Depression is also linked to sleep disturbance[227], and in general might be under-recognized and under-treated in patients with SCD. Asthma is common: it occurs in at least 25% of children with SCD and is associated with increased incidence of acute pain events, acute chest syndrome and early death[174] Venous thrombosis has been reported in up to 25% of patients with SCD, and could be due to the commonly observed activation of the haemostatic system[228].

[H1] Quality of life

Generic health-related quality of life (HRQOL) instruments (for example, the 36-item short-form (SF-36) for adults and the Pediatric Quality of Life Inventory (PedsQL) for children)[229,230] measure physical, emotional and social functioning and enable the comparison of patients with SCD with healthy individuals. Disease-specific measures have better specificity for detecting differences within a population of patients with SCD and are also designed to detect changes in HRQOL over time such as the PedsQL™ Sickle Cell Disease module for children with SCD[231].

Both adults and children with SCD have substantially impaired baseline HRQOL (**Figure 7**) [198,232]. Compared with healthy individuals, patients with SCD have impaired HRQOL in nearly every domain, especially within the areas of pain, fatigue and physical functioning[233,234]. Adolescents and adults report poor sleep quality, moderate levels of fatigue and that sleep quality mediates the relationship between pain and fatigue[235]. The baseline physical functioning HRQOL domain, of many patients with SCD is worse than or comparable with that of patients with other chronic diseases, such as cancer, cystic fibrosis or obesity[236].

Acute complications, such as an acute vaso-occlusive pain crisis, are significantly associated with worse HRQOL than at baseline[237]. Children report substantial problems with physical functioning, pain and sleep during and immediately following vaso-occlusive crises[238]. Daily pain can affect the ability to attend school or work[239,240] and is predictive of worse HRQOL in adults[241]. Nearly one-third of adults report pain almost every day and over half of the patients have pain 50% of the time[240].

[H2] EFFECT OF TREATMENT ON HRQOL

Adult patients who had a favourable response to hydroxycarbamide had better general health and reduced pain than those who received placebo or had a low response to treatment[242]. Similar results were observed in children who received hydroxycarbamide [243] or chronic red blood cell transfusion therapy[244]. As more experimental drugs for patients with SCD are tested in clinical trials, it is imperative to measure the effect of these new therapies on patient's HRQOL.

[H1] OUTLOOK

The widely implementation of affordable interventions including neonatal diagnosis, penicillin prophylaxis and vaccination (which led to substantial reductions in mortality among children with SCA <5 years of age in high-income countries) could prolong the lives of ~5 million newborn babies with SCA by 2050[17]. Similarly, large-scale screening and treatment programmes could save the lives of up to 10 million newborn babies with SCA globally, most of them in sub-Saharan Africa[17,39].

[H2] SCREENING

Screening for SCD and related conditions is essential in Africa, where the incidence is highest. However, the implementation of universal newborn babies screening programmes remains a major economical and public health challenge. African communities and governments should also develop culturally acceptable programmes for screening adults for family planning purposes. The development of new accurate and affordable rapid diagnostic tests would offer a long-awaited point of care screening option for low-income and middle-income countries. Clinical validation of such tests showed that they can reliably detect the β^S and β^C alleles with high specificity and sensitivity[245]. These tests could be used as a large-scale first screening step before confirmation of diagnosis by HPLC or IEF, which will be necessary to identify individuals who also have thalassaemia or other Hb variants.

[H2] TREATMENT

In the short term, the identification of ways to enhance the use of proven therapies, such as haematopoietic stem cell transplantation and hydroxycarbamide, is the quickest route to improve management. Nevertheless, questions remain about the long-term efficacy of hydroxycarbamide, ways to improve adherence to hydroxycarbamide therapy and possible development of antibacterial resistance in children with SCD under long-term penicillin prophylaxis. Owing to the complexity of SCD and the range of possible complications, a multi-drug approach will probably be used by health care providers. However, the drug development is a time-consuming process; thus, multi-drug treatments will probably be available only in the mid-term or long-term. Future work to understand the HRQOL of patients over time and outside of the medical system and the effect of therapy on HRQOL is needed to provide tailored care and maximize the HRQOL[246].

Gene therapy has been seen as a promising cure for SCD since the mid-1990s. Lentiviral vectors have been developed to insert gamma or modified beta globin genes that have been engineered to reduce sickling into haematopoietic stem cells; these vectors are now in clinical trials[247] and have yielded a promising initial result[248]. Newer gene editing approaches based on zinc finger nucleases and transcription activator-like effector nucleases have been designed and tested for proof of principle in SCD[249]. The development of clustered regularly interspaced short palindromic repeats (CRISPR) techniques, which enable the precise replacement of a specific region of DNA, is another promising gene therapy approach for SCD, currently only tested in mice[250] and cultured human cells[251] until the multi-year regulatory process is cleared for human trials. However, many ethical issues need to be resolved

before these techniques can be used in human patients: long-term follow-up trials will be needed to confirm the safety and sustainability, and the accessibility of gene therapy in high-burden, low-income areas needs to be addressed. Although some of these current gene therapy strategies are potentially curative, many of them only aim to ameliorate disease severity.

[H3] NEW DRUGS

In the United States, the decision of the FDA Division of Hematology Products to consider the development of new SCD treatments as a top priority and grant orphan drug status or "fast track" designation to several drugs and biological products has facilitated investments form pharmaceutical companies. Many products that target one or more of the mechanisms that contribute to the disease process (for example, by boosting HbF levels or countering oxidative stress) are currently in Phase II or Phase III trial[252] (**Table 1)**. A large clinical trial of an anti-platelet agent, prasugrel failed to significantly reduce vaso-occlusive crisis episodes in children with SCA[151], but P-selectin blocking approaches are promising, to prevent[146] and to reduce duration and severity[253] of vaso-occlusive crisis episodes. Enrolment in SCD trials remains challenging: a systematic review of 174 SCD interventional trials closed to enrolment showed that 57% of them terminated owing to lowenrolment[254]. However, the recent completion of a series of large, multicentre, multinational clinical trials demonstrate that the SCD patient and provider community are eager to collaborate with the pharmaceutical industry to find effective new treatments[146,147,151,253,255]. The prospects for new treatments in SCD has never looked better.

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Author contributions

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Competing interests

G.J.K. is listed as a coinventor on a patent application by the NIH for the formulation of topical sodium nitrite (PCT/US2015/060015), receives research support from Bayer Pharmaceuticals, and has received research support from AesRx, LLC and personal consulting fees (honoraria) from Novartis and Bioverativ, outside the submitted work. The University of Pittsburgh received support for G.J.K.'s salary to serve on the steering committee for a clinical trial by Mast Therapeutics, Inc. F.B.P. reports personal fees (honorarium) from Novartis, outside the submitted work. L.K., W.R.S, J.A.P., D.J.W., F.F.C. and E.V.P. declare no competing interests. Editor's note: All other authors have chosen not to declare any competing interests.

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Box 1. Roadmap to screening programmes in the Unites States

The National Sickle Cell Anemia Control Act (Public Law 92-294) was signed into law in 1972 in response to a Presidential initiative and Congressional mandate[256]. It provided for voluntary SCD screening and counselling, education programmes for health professionals and the public and research and training in the diagnosis and treatment of SCD. Because of this legislation, a national broad-based programme of basic and clinical research was established at the National Institutes of Health (NIH) and coordinated across federal agencies. The Comprehensive Sickle Cell Centers were the major component of this programme; ten Centers were established in hospitals and universities located in geographic areas with large at-risk populations. These Centres provided an integrated programme of research and care of patients with SCD and also emphasized prevention, education, early diagnosis and counselling programmes supported by the NIH. The establishment of treatment guidelines and protocols standardized treatment across the country. . The centres gradually shifted toward basic and clinical research, and the NIH Centres programme was disassembled in 2008.

Box 2. Screening in Ghana

The screening programme in Ghana is designed to be universal and include neonates born at both public and private birth facilities, and "well-baby", free immunization clinics (that is, public health clinics where babies are brought to receive free immunizations) for babies who were not screened at birth or were referred from facilities where the screening is not available[121]. Babies with possible SCD are referred to a treatment centre, where a second sample is obtained to confirm the initial screening results. Babies with SCD are enrolled in a comprehensive care programme that includes penicillin and anti-malarial prophylaxis, folic acid supplementation and parental education about management of SCD. Ghana's National Health Insurance Authority funds newborn babies screening programmes as part of the mandated free care for children <5 years of age. By the end of 2015, >400,000 newborn babies were screened for SCD and related conditions. Of the 6,941 newborn babies who were diagnosed with SCD, 80% had been successfully followed up, and 70% of them registered at the Kumasi Center for SCD, which had been established for the pilot screening programme (K.O.-F., unpublished observations). However, follow-up is challenging, as 80% of mothers of babies with SCD initially failed to return for results and had to be reached at their homes and irregular government funding can cause intermittent shortages of laboratory supplies. Limited funding has stalled the national scale up of the free screening program, which currently reaches only 4.2% of the 850,000 annual number of neonates.

Box 3: Screening in Brazil

The Newborn Screening Program in Brazil was implemented as an official program of the Federal Government in 2001, but a few statewide programmes were already in place. As of 2017, the National Program for Newborn Screening (PNTN) is available to all 26 states of the country, although the coverage is highly variable (for example, in 2016, it was almost 100% of hospitals in the state of Minas Gerais and ~55% in the state of Amapa)[257].

The newborn babies screening programmes enabled the analysis of the survival of children with SCD. In the state of Minas Gerais >3.6 million newborn babies were screened between 1998 and 2012 and >2,500 children were diagnosed with SCD. During the 14-year study period, the mortality rate was 7.4%. The main causes were infection (45%) and acute splenic sequestration (14%).[257] In another study in the state of Rio de Janeiro, >1.2 million newborn babies were screened between 2000 and 2010, and 912 had SCD. The mortality was 4.2% during the 10-year period and the main causes were acute chest syndrome (36.8%), sepsis (31.6%) and splenic sequestration (21.1%).[26]

Figures

Figure 1: Genetic alterations in the haemoglobin subunit β gene (*HBB*). Normal haemoglobin A (HbA) is formed by 2 α globin proteins and two β globin proteins, the latter of which is encoded by *HBB*. The sickle Hb (HbS) allele β^S is a *HBB* allele in which an adenine to thymidine substitution results in the replacement of glutamic acid (Glu) with valine (Val) at position 6 in the mature β-globin chain. Sickle cell disease (SCD) occurs when both *HBB* alleles are mutated and at least one of them is the β^S allele. Deoxygenated (not bound to oxygen) HbS can polymerize and HbS polymers can deform the erythrocyte. Individuals with one β^S allele have the sickle cell trait (HbAS), but not SCD; individuals with sickle cell anaemia (SCA), the most common SCD genotype, have two β^S alleles (β^S/β^S). Other relatively common SCD genotypes are possible. Individuals with the HbSC genotype have one β^S allele and one allele with a different nucleotide substitution (*HBB* Glu6Lys, or β^C allele) that generates another structural variant of Hb, HbC. The β^C allele is mostly prevalent in West Africa or individuals with ancestry from this region[16]. HbSC disease is a condition with generally milder haemolytic anaemia and less frequent acute and chronic complications than SCA, although retinopathy and osteonecrosis (also known as bone infarction, in which bone tissue is lost owing to interruption of the blood flow) are common occurrences[258]. The β^S allele combined with a null *HBB* allele ($Hb\beta^0$) that results in no protein translation results in $HbS\beta^0$–thalassaemia, a clinical syndrome indistinguishable from SCA except for the presence of microcytosis (a condition in which erythrocytes are abnormally small) [259]. The β^S allele combined with a hypomorphic *HBB* allele ($Hb\beta^+$) (with a decreased amount of normal beta globin protein) results in $HbS\beta^+$-thalassaemia, a clinical syndrome generally milder than SCA owing to low level expression of normal HbA. Severe and moderate forms of HbSβ-thalassaemia are most prevalent in the eastern Mediterranean region and parts of India, whereas mild forms are common in populations of African ancestry. Rarely seen compound heterozygous SCD genotypes include HbS combined with HbD, HbE, HbO^{Arab} or haemoglobin Lepore (not shown)[260].

Figure 2: Map of the estimated numbers of births with sickle cell anaemia. Estimated numbers of births with sickle cell anaemia per 100,000 births per country in 2015. Estimates are derived from prevalence data published in[14]. Birth data for 2015-2020 were extracted from the 2017 Revision of the United Nations World Population Prospects database available online at https://esa.un.org/unpd/wpp/Download/Standard/Fertility/.

Figure 3 HbS polymerization and erythrocyte deformation

Long polymers of sickle haemoglobin (HbS) align into fibres, which then align into parallel rods. The polymer has a helical structure with 14 HbS molecules in each section[41,54,261]. HbS polymerization depends on many factors, including HbS concentration, partial pressure of oxygen (pO_2), temperature, pH, 2,3-diphosphoglycerate (2,3-DPG) concentration and the presence of different Hb molecules[262-264]. The basic concept of HbS polymerization kinetics is the double nucleation mechanism. Before any polymer is detected, there is a latency period (delay time) in which deoxygenated HbS molecules form a small nucleus, which is followed by rapid polymer growth and formation[265,266]. Free cytoplasmic heme can increase the attraction of the HbS molecules and the speed of nucleation and polymer formation[267].

Cation homeostasis is abnormal in sickle erythrocytes, leading to the dehydration of cells. Potassium loss occurs via the intermediate conductance calcium-activated potassium channel protein 4 (also known as Gardos channel) and potassium chloride (KCl) cotransporter 1 (KCC1), KCC3 and/or KCC4) [268,269]. Plasma adenosine can also reprogram the metabolism of the erythrocyte, altering sphingosine-1-phosphate . ADORA2B, adenosine receptor A2b; AE1, band 3 anion transport protein.

Figure 4: Mechanisms in sickle cell disease.

Damage and dysfunction of the erythrocyte membrane caused by sickle haemoglobin (HbS) polymerization leads to haemolysis. Oxidized membrane proteins reveal antigens that bind to existing antibodies, and membranes expose phosphatidylserine; both mechanisms promote phagocytosis of erythrocytes by macrophages, a pathway of extravascular haemolysis. Intravascular haemolysis releases the contents of erythrocytes in the plasma. Hb scavenges nitric oxide (NO), arginase depletes the L-arginine substrate of NO synthase and asymmetric dimethylarginine (ADMA) inhibits NO synthase. Reactive oxygen species further deplete NO, leading to vasoconstriction and vascular remodelling, especially in the lung. Adenine nucleotides and NO deficiency promote platelet activation and activation of blood clotting proteins. Heme and other danger associated molecular pattern (DAMP) molecules activate the innate immune system. Ligand-bound toll-like receptor 4 (TLR4) and TLR2 activate monocytes and macrophages to release inflammatory cytokines, which promote an inflammatory state and activation of endothelial cells. TLR4 activation on platelets promotes their adhesion to neutrophils, which in turn release DNA to form neutrophil extracellular traps (NET). Circulating blood cells adhere to each other and to activated endothelium, contributing and potentially even initiating vaso-occlusion. In post-capillary venules, activated endothelial cells that express P-selectin and E-selectin can bind rolling neutrophils. Activated platelets and adhesive sickle erythrocytes can adhere to circulating or endothelium-bound neutrophils and form aggregates. Sickle erythrocytes might also bind directly to the activated endothelium. The figure only shows some examples of the complex and redundant receptor-ligand interactions involved in the adhesion of circulating cells to the damaged endothelium and exposed subendothelium.

Figure 5: SCD clinical complications. Acute complications bring the patient to immediate medical attention; pain is the most common acute complication. As patients age, chronic complications produce organ dysfunction that can contribute to earlier death. Complications of pregnancy include pre-eclampsia, intra-uterine growth restriction, preterm delivery and perinatal mortality.

Figure 6 Age-distribution of chronic SCD complications. Development of clinical complications in 5,100 patients with SCD identified in the California Hemoglobinopathy Surveillance Program[270].

Figure 7. Health-related quality of life. Physical functioning scores measured using the SF-36 and the PedsQL generic core scales in healthy individuals and patients with chronic disease.[236, 271] Scores range from 100, representing the best health-related quality of life, to 0. Specific areas represented in physical functioning scores include the ability to perform all types of physical activities, such as running, walking for a short distance, lifting heaving objects and bathing without help.

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1711 **Table 1. Emerging treatment approaches for sickle cell disease.**

Therapy (previous name)	Mechanism	Advantages	Limitations	Refs.
		FDA approved		
L-glutamine	Increases NADH levels and, as a result, cellular antioxidant activity	Oral formulation available; reduced the frequency of acute complications	Phase III trial results not yet published	[272]
		Phase III study		
Rivipansel (GMI-1070)	Pan-selectin inhibitor	Can reduce the duration of pain crises, shorten hospital stays and decrease the amount of opioid pain medication	Currently available only in intravenous formulation to be used at the time of pain episodes.	[253]
Hydroxycarbamide	Increases expression of HbF	Reduces frequency of acute pain events, acute chest syndrome and transfusions in infants and adults	Disproportionate perceptions of carcinogenicity, teratogenicity and reduced fertility	[143,150]
Prasugrel	Platelet inhibitor	Hypothesized to reduce the duration of vaso-occlusive crisis; seems to be well-tolerated at both therapeutic and supratherapeutic doses	Phase III study results not significant	[273]
Vepoloxamer (MST-188)	Enhances microvascular blood flow	Hypothesized to reduce the duration and severity of acute pain crises	Phase III study results showed no effect (press release)	[274]
L-arginine	NOS substrate	Significantly reduced the severity of vaso-occlusive crisis in Phase II studies **[Au:OK?]**	Phase III trial results not yet available.	[275,276]

N-acetylcysteine	Antioxidant	Oral administration	Phase III study results showed no effect	[277]
Magnesium sulfate	Multimodal	Vasodilator, anti-inflammatory and pain reliever activities	Phase III study results showed no effect	[278]
Transfusions for silent cerebral infarcts	Erythrocyte transfusion	Significantly reduced the incidence of the recurrence of ischaemic stroke in children	Cumbersome to move into general practice	[211]
Transfusions for stroke prevention	Erythrocyte transfusion	Significantly reduced incidence of first stroke in children with high cerebral artery blood flow	Follow-up study showed that it was not safe to stop regular transfusions after 30 months	[206,207]
Transfusions changing to hydroxycarbamide	Increases expression of HbF	Efficacious for primary stroke prophylaxis	Not clearly superior to chronic transfusion for secondary stroke prophylaxis	[209,279]
		Phase II study		
Crizanlizumab (SelG1)	P-selectin inhibitor	Reduced the incidence of acute complications by 45-63%.	Monthly intravenous infusions required	[146]
Inhaled NO	Pulmonary vasodilator	Provides NO to correct decreased bioavailability	Phase II trial showed no effect on the duration or severity of vaso-occlusive pain crisis	[280]
Sildenafil	PDE5A inhibitor	FDA approved for pulmonary hypertension and erectile dysfunction	Phase II trial terminated early owing to increased frequency of acute pain events	[281]
Sanguinate*	Improves tissue oxygen levels	Hypothesized to prevent vaso-occlusive crisis and leg ulcers.	Limited data	[282]
Sevuparin	Enhances microvascular	Might decrease erythrocyte adhesion and favour normal blood flow, and reduce the	Limited data	[283]

(DF02)*	blood flow	risk of vaso-occlusion.		
GBT440*	HbS polymerization inhibitor	Well tolerated; proof of concept with improved oxygen delivery to tissues and marked reduction in circulating sickle erythrocytes	Limited data	[284]
		Phase I study		
Pomalidomide	Increases fetal haemoglobin	Well tolerated; increases HbF and total Hb levels; anti-inflammatory effects	Limited data	[285]
IMR-687*	PDE9A inhibitor	Preclinical data indicate decreased sickling, neutrophil adhesiveness and vaso-occlusion	Limited data	[286]
SCD-101	HbS polymerization inhibitor	Natural product	Limited data	[287]
Gene insertion	Lentiviral vectors	Insertion of genes encoding anti-sickling engineered β globins	Unknown long-term risks; unclear if curative or only ameliorative	[247]
		Preclinical study		
Genome editing	Programmable nucleases	Methods include zinc finger nucleases, , transcription activator-like effector nucleases and CRISPR/Cas9	Unknown long-term risks; potential cure or disease amelioration, depending on strategy	[249]

Adapted from refs[254,288]. * granted FDA orphan drug status

Cas9, CRISPR-associated endonuclease cas9; CRISPR, clustered regularly interspaced short palindromic repeats; HbF, foetal haemoglobin; NADH, reduced nicotinamide adenine dinucleotide; NO, nitric oxide; NOS, nitric oxide synthase; PDE5A, cGMP-specific 3',5'-cyclic phosphodiesterase; PDE9A, high affinity cGMP-specific 3',5'-cyclic phosphodiesterase 9A

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ToC blurb

Sickle cell disease includes genetic conditions that are caused by mutations in one of the genes encoding haemoglobin. Mutant haemoglobin molecules can polymerize, causing the red blood cells to acquire a characteristic crescent shape that gives the disease its name.