

# Sickle Cell Disease

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## Abstract

Sickle cell disease and its variants constitute the most common inherited blood disorders affecting millions of individuals worldwide. Significant information regarding the nature of the genetic mutations and modifier genes that result in increased or decreased severity of the disease are available. In recent years, detailed data regarding molecular genetics, pathophysiology, mechanisms for the development of symptoms and side effects of sickle cell disease have been published. The relationship of physiological changes, cellular interactions, coexisting coagulation disorders, effects of association with other genetic disorders and a number of intervening factors have been explored. New techniques for pre-conception, prenatal, in utero, and neonatal screening are available. Means for prediction of the severity of the disease, clinical course of the disorder, and prevention of some of its major complications have been developed. The effects of psychosocial and environmental factors have been explored. Various therapeutic strategies including bone marrow and stem cell transplantation are currently employed in the treatment of patients with sickle cell disease. Recent progress in understanding the molecular pathways controlling mammalian erythropoiesis and globin switching, as well as advances in genome engineering, particularly the gene-editing techniques, have opened a venue for genetic-based treatment of the disease. Currently, sickle cell disease is often associated with a high rate of complications and mortality. The development of new pharmacological agents, methods for gene therapy, and alterations and modification of the coexisting genetic factors and modifiers for treatment of the disease are encouraging.

## KEYWORDS

sicklecelldisease,genetics,etiology,pathophysiology,symptoms,screening,diagnosis,complications,treatment,coagulation,inflammatory factors, modifiers,genetherapy, transplantation.

## INTRODUCTION

In 1910, sickle cell disease burst onto the Western medical scene as a “strange” or, as Herrick termed it, a “new, unknown disease.” Physicians were intrigued by the sickled appearance of the red cells in this disorder, and case reports and analytical papers detailing the clinical features of this disorder appeared to almost always involve people of color. The disease then became known as a “black disease.” Not until 1949, however, was the molecular nature of sickle cell discovered. In 1958, Ingram discovered the genetic basis of the disease and demonstrated that the disease originated from the substitution of a valine for glutamic acid at the sixth amino acid position of the hemoglobin beta chain. This amino acid

substitution, now known to be the result of a single point mutation of the hemoglobin gene, produces profound changes in the behavior and conformation of the hemoglobin molecule in individuals affected by the disease. In 1927, Hahn and Gillespie had reported on the mechanism of sickle formation, observing that the sickle hemoglobin in its deoxygenated state assumed the characteristic shape, the sickle, that gives the disorder its name. Cells containing deoxygenated hemoglobin not only formed this rigid shape but also were dehydrated, had abnormal cell surface and distinct migratory characteristics, were sticky and prone to adhesion, and had abnormal rheologic properties. Clinically, not only did patients with sickle cell Clinically, not only did patients with sickle cell disease experience repeated painful episodes (crises), but because of recurrent episodes of vaso-occlusion, they ultimately suffered chronic organ damage. Physicians noted a paucity of individuals who survived into their adult years Sickle cell disease, one of the most common inherited diseases worldwide, is now understood to be a disorder of global importance and economic as well as clinical significance. Those affected by the disease live in areas of sub-Saharan Africa, the Middle East, India, the Caribbean, South and Central America, some countries along the Mediterranean Sea, as well as in the United States and Europe. The disease has, at times, through forced and unforced migration, been introduced to areas in which it was not endemic In the United States, 80,000-100,000 individuals are affected by the disorder; worldwide, more than 300,000 children are estimated to be born annually with sickle cell disease. This number includes approximately 3,000 children born with the disease each year in the United States. Since the 1980s, novel approaches for the treatment of sickle cell disease have included the introduction of penicillin prophylaxis for children with sickle cell, the institution of newborn screening programs, and the use of transcranial Doppler screening for detection of cerebral vasculopathy and stroke prevention. Hematologists have long recognized the need for better treatments of sickle cell. Optimally, a treatment approach was needed that did not just address pain or treat and prevent sequelae of the disease (eg, susceptibility to infection from asplenia). What was needed instead was a treatment approach that worked by undercutting the pathophysiology of the disease. Research efforts previously concentrated on understanding the pathogenesis of the disease rather than on providing greater relief for the patients having the disorder. Progress in arriving at satisfactory treatment of individuals with sickle cell has often seemed to be a slow, halting process. Also, funding for research of sickle cell disease lagged behind that of other genetic diseases, fueling a suspicion that racial bias prevented significant outlays of moneys for study of the disorder. The innovations enumerated above did result in stepwise improvements in survival, so the median life expectancy for those with homozygous disease is now into the fourth and fifth decades. Beyond hydroxyurea, which was introduced into clinical practice in the 1980s for adults, few new drugs have been investigated or placed on the market for the treatment of the disorder until recently<sup>1-5</sup>.

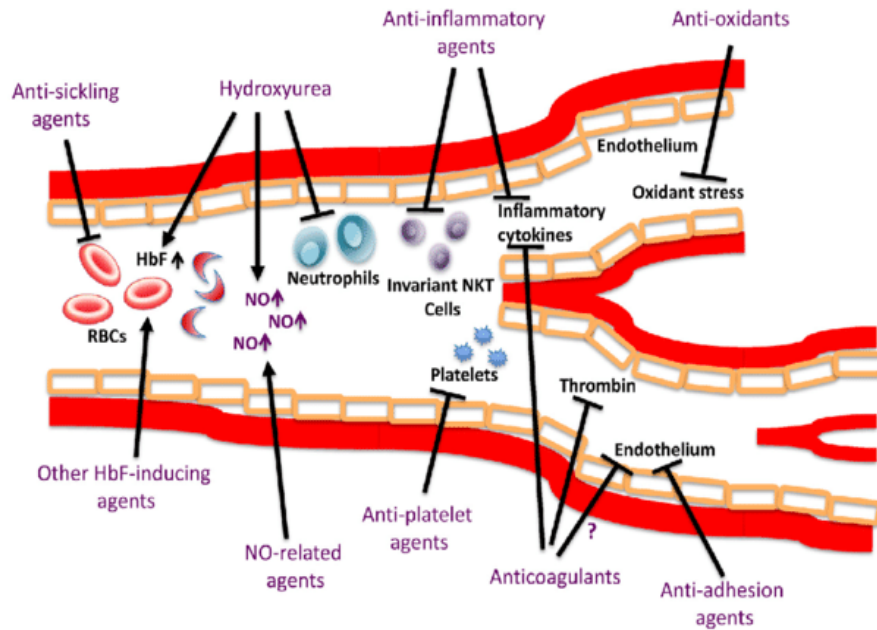
### **Epidemiology, Distribution, Incidence, and Prevalence**

Sickle cell disease and its variants constitute the most common inherited human blood disorders affecting millions of individuals worldwide. While statistical reports vary, globally, approximately 4.4 million individuals have sickle cell disease. Worldwide, each year, approximately 300,000 infants are born with this disorder, including an estimated 3000 in the United States, where it is estimated that 80,000 to over 100,000 individuals have this disease . According to the Centers for Disease Control and Prevention (CDC), one in 365 Black and one in 13,600 Hispanic Americans have SCD. The disease is most prevalent in Sub-Saharan Africa with approximately 75–80% of cases occurring in individuals with

a background from that region. Sickle cell disease is also prevalent in parts of India, the Middle East, Eastern Mediterranean, Arabian Peninsula, Sicily, Greece, southern Turkey, and in individuals with African ancestry living in North and South America and other parts of the world. It is believed that due to the “protective effect” and the survival advantage of individuals heterozygote for sickle cell, the disease is prevalent in areas where malaria is endemic. It is postulated that compared to the survival of noncarriers, carriers of the recessive SCD gene have a survival advantage if affected by malaria and are more likely to reach reproductive age. Due to this fact, prevalence of SCD in Africa, where malaria is a major life-threatening disease, is high. Migration of West and Central Africans within Africa has led to the introduction of SCD to South Africa. While SCD has a worldwide distribution, in some countries, it constitutes a rare disorder, resulting in diagnostic and management problems. It is worthwhile to note that in 2006, the World Health Organization (WHO) recognized SCD as a public health priority<sup>6-8</sup>.

### **Pathophysiology**

The development of effective therapies for SCD depends on an adequate understanding of its pathophysiology. Although the pathophysiology of SCD is complex and involves multiple pathways, the primary event is due to the polymerization of HbS following deoxygenation. The rate and extent of polymer formation depends on the degree and duration of HbS deoxygenation, presence of fetal hemoglobin (HbF), and the intracellular concentration of HbS. Clinical manifestations of SCD appear to be driven by two major pathophysiological processes: vaso-occlusion with ischemia-reperfusion injury and hemolytic anemia. Vaso-occlusion occurs because of adhesive interactions of leukocytes and sickle RBCs with the endothelium causing microvascular obstruction and subsequent tissue ischemia. These episodes of vascular obstruction are followed by the restoration of blood flow, which promotes further tissue injury by reperfusion. The inflammatory cascade resulting from ischemiareperfusion is amplified by the activation of CD1d-restricted invariant natural killer T (iNKT) cells. The release of free plasma hemoglobin following intravascular hemolysis results in direct scavenging of nitric oxide (NO), as well as the generation of reactive oxygen species, powerful scavengers of NO. NO is usually produced by the endothelium and regulates basal vasodilator tone as well as inhibits the activation of platelets and the coagulation system and the transcriptional expression of nuclear factor  $\kappa$ B (NF $\kappa$ B)-dependent adhesion molecules, such as vascular cell-adhesion molecule-1, intercellular cell-adhesion molecule-1, and selectins. HbS polymerization as well as its multiple downstream consequences, including endothelial cell injury, endothelial dysfunction, increased oxidant stress, inflammation, and coagulation and platelet activation, are therapeutic targets in SCD (Figure 1). SCD has been dichotomized into two overlapping sub-phenotypes: viscosity-vaso-occlusion (higher hemoglobin levels, possibly increased blood viscosity, and complications such as osteonecrosis, acute chest syndrome, and acute pain crisis) and hemolysis-endothelial dysfunction (increased hemolysis with lower hemoglobin levels and higher levels of hemolytic markers, including reticulocyte count and serum lactate dehydrogenase, and complications such as leg ulcers, priapism, stroke, and possibly pulmonary hypertension). While somewhat simplistic, this classification is useful to understand the pathobiology of disease complications and may provide guidance on the effects of therapies on disease-related complications. The pathophysiology of SCD has been reviewed more extensively elsewhere<sup>8-10</sup>.



**Figure 1** .Potential targets of pharmacologic agents in sickle cell disease. Hbf , hemoglobin F; NKT, natural killer T-cells; NO, nitric oxide; RBCs, red blood cells.

### Diagnosis

1. Blood test
2. Hb level count
3. Check the type of haemoglobin

### Risk factors

Individuals with sickle cell disease, homozygous for the  $\beta^S$ , are most likely to have characteristic common symptoms and complications of the disease. During early infancy, sickle cell disease is basically asymptomatic. Manifestations of the disease begin with a decline in the fetal hemoglobin. Later in life, a number of intervening factors can alter the symptomatology of sickle cell disease. Some of the common manifestations of the disease include hemolytic anemia and chronic low-level pain, mainly in bones and joints. Intermittent vaso-occlusive crises are common. This disorder is also associated with several complications including hand-foot syndrome, acute chest syndrome, splenic sequestration, loss of vision, growth retardation, leg ulcers, deep vein thrombosis, infections, damage to various tissues and organs including liver and bones. Disorders of the heart, kidneys, liver, gallstones, priapism, and most importantly, stroke and other central nervous system (CNS) complications are common. Approximately 10 percent of children with SCD can have asymptomatic stroke. Ischemic stroke, sinovenous thrombosis, posterior leukoencephalopathy, and acute demyelination can result in additional complications including seizures, learning problems, physical disabilities, and coma. Painful crisis due to vaso-occlusion and bone infarction frequently occurs. While acute pain crisis is most often managed at home, it is the common reason for patients with this disease to seek medical attention and constitutes the most frequent cause for emergency room visits and hospitalization. Dactylitis or bony infarction of digits, resulting in pain and swelling of fingers or toes, are often seen in infants. Recurrent vaso-occlusions and infarctions result in avascular necrosis of bones and articular surfaces, especially heads of long bones. Osteopenia and osteoporosis are frequent findings in sickle cell disease and can cause

vertebral collapse and chronic back pain. Increased rates of bone infarction have been reported to be influenced by the higher levels of hematocrit and the concomitant presence of  $\alpha$ -thalassemia trait. The latter, however, is based on a small number of patients and not statistically significant data. The most common symptoms and morbidities associated with SCD include pain crises, acute chest syndrome, cerebrovascular accidents, and splenic and renal dysfunction<sup>4-5</sup>.

### **Cardiac Complication**

Cardiac complications are common in SCD and can cause significant morbidity and mortality. Chronic anemia results in increased cardiac output, but only a minimal elevation of heart rate. Anemia causes an increased left ventricular stroke volume with significant dilation of the left ventricle, and eventually, development of eccentric hypertrophy and myofibers resulting in an increased left ventricular mass and elongation, as well as diastolic dysfunction. Pulmonary hypertension and right ventricular dysfunction have also been reported<sup>11-13</sup>.

### **Genitourinary Complications**

Patients with sickle cell disease may have a wide variety of renal dysfunctions. Relative hypoxia in the renal medulla and decreased blood flow in the vasa recta can result in sickle formation of the erythrocytes causing veno-occlusion and infarction. The manifestations of sickle cell nephropathy can include impairment of urinary concentrating ability, some degree of hypophosphatemia and increased creatinine clearance, and impairment of urinary acidification and potassium excretion. Hematuria, proteinuria, tubular disturbances, and chronic kidney disease are common in sickle cell disease patients. Painless hematuria is often seen and is usually benign. Tubular functional defects and proteinuria may be signs of the development of chronic sickle cell nephropathy. Priapism can be a very painful and serious complication of sickle cell disease. The probability of having at least one episode of priapism by age 20 can be 89%. While conservative therapy is often sufficient, treatment with hydroxyurea, etilefrine, pseudoephedrine, leuprolide, sildenafil, and other agents are deemed to be helpful. The role of other treatments such as nitric oxide is uncertain. Possible use of polyethylene glycol-modified ADA (PEG-ADA) enzyme therapy and A(2B)R antagonists as a future novel treatment for priapism have been suggested. If an episode, despite hydration and analgesic therapy persists, intracavernosal aspiration and instillation of an  $\alpha$ -agonist and surgery may be considered<sup>14</sup>.

### **Hepatobiliary Complications**

Hepatobiliary system is commonly affected in SCD. The “sickle cell hepatopathy” can have a broad spectrum of manifestation ranging from benign hyperbilirubinemia to liver failure. Hyperbilirubinemia due to sickling process can lead to ischemia, sequestration and cholestasis. Cholelithiasis is common in sickle cell disease affecting 15% of children with this disorder before 10 years of age and 80% of patients over age 30. While the best option for asymptomatic cholelithiasis is not clear and should be decided on an individual patient’s basis, laparoscopic cholecystectomy appears to be the treatment of choice in patients with clinically symptomatic disease<sup>15</sup>.

### **Bases of Treatment**

Currently, in most cases, goals for the treatment of sickle cell disease include prevention and control of symptoms and complications. Early intervention and treatment of the complications of sickle cell disease



is essential. This includes obtaining timely and serial transcranial doppler ultrasounds for the prevention of stroke, detection and treatment of pulmonary hypertension, and detection and treatment of complications and damages to the various organs and systems which are associated with this disease. Preventive treatments with hydroxyurea, P-selectin inhibitors, e.g., crizanlizumab hemoglobin oxygen-affinity modulators, e.g., Voxelotor are commercially available. Hydroxyurea increases total and fetal hemoglobin in SCD, thus reducing gelation and sickling of erythrocytes. It also reduces the levels of circulating leukocytes, which decreases the adherence of neutrophils to the vascular endothelium. Voxelotor is a hemoglobin S polymerization inhibitor designed to reversibly bind to hemoglobin in order to stabilize the oxygenated hemoglobin state<sup>16</sup>.

## CURES FOR SICKLE CELL DISEASE

### Stem Cell Transplantation

The only cure available to patients with sickle cell disease is stem cell transplantation. However, the selection of patients who should benefit from this treatment modality is controversial. Transplant has been performed, for the most part, in patients who have suffered a stroke, have had multiple episodes of acute chest syndrome, or have had recurrent vaso-occlusive crises ( $\geq 3$  episodes requiring hospitalization per year), ie, patients considered to have the worst disease severity. Controversies have arisen not only about whom to transplant but also about the optimal age to transplant, source of donor cells, and type of conditioning regimen. Most stem cell transplants thus far have relied upon myeloablative conditioning regimens and have been bone marrow-derived with human leukocyte antigen (HLA)- matched sibling donors as the source of stem cells. But the probability of an individual having a matched sibling donor is only 16%-20% among minorities if an 8 of 8 allele match is sought. The effort to expand the availability of transplant for most patients with sickle cell disease has led to consideration of alternative donor sources, such as cord blood, matched unrelated, and haploidentical cells. Gluckman et al conducted a survey of 1,000 recipients of HLA-identical sibling transplants from European, American, and non-European centers. Sixty percent of patients underwent myeloablative conditioning, and the unadjusted overall survival rate after 5 years and event-free survival rate were 92.9% and 91.4%, respectively. Transplant led to stabilization of organ function, gradually ameliorated complications of sickle cell disease such as cardiovascular and pulmonary dysfunction, and reduced the occurrence of vaso-occlusive episodes. In another series, results from HLA-identical sibling transplants after myeloablative conditioning with antithymocyte globulin were reported. The event-free survival rate for sibling transplants after myeloablative regimens was approximately 95% in this series. While myeloablative conditioning has remained the standard of care for hematopoietic stem cell transplantation, it has been associated with toxicities that have included venoocclusive disease of the liver and neurotoxicities such as seizures, stroke, and brain hemorrhage. Late effects of transplant such as growth failure, hypogonadism, sterility, and secondary malignancies have also been reported. The median age for transplantation has been 9-10 years; individuals who are older have not fared as well, with a lower probability of survival in general and of graft-vs-host-disease (GVHD)-free survival in particular. Attempts at decreasing the toxicities associated with transplantation have resulted in the use of less-rigorous conditioning regimens (reduced-intensity conditioning regimens). For these transplants, the goal became producing a state of mixed chimerism in which recipient marrow is incompletely replaced by donor cells, producing in some instances a trait-like phenotype. These regimens have been better tolerated, especially in patients with preexisting comorbidities, and have resulted in an 86%-90%

disease-free survival rate. Indications of what the lower limit of red cell donor chimerism is to allow improvement of disease manifestations have varied. In 2017, Fitzhugh and colleagues published a paper in which they stated that chimerism of 20% is necessary to abrogate the sickle phenotype. However, the earlier experience of Walters and coauthors cited results in which one individual with as few as 11% donor cells expressed a hemoglobin S level of 7% and ceased to have a transfusion requirement; he also did not have symptoms consistent with sickle cell disease any longer. One significant problem associated with reduced-intensity transplantation remains the higher likelihood of loss of donor cells or engraftment failure. The search for alternative sources of stem cells has also led to the use of unrelated donors. Unrelated donor marrow transplants have had less success, with 1- and 2-year event-free survival rates of 76% and 69%, respectively, and overall survival of 86% and 79%, respectively. The rate of GVHD was relatively high (62%), and more GVHD-related deaths occurred than would be ordinarily seen with related donors. Unrelated cord blood has also been proposed as a source of donor cells, but the graft failure rate in one study was fairly high (52%), and the overall survival was 94%. In one trial utilizing a reduced-intensity conditioning regimen prior to transplantation with unrelated cord blood, a graft failure rate up to 63% was observed, leading the authors to conclude that donor engraftment needs to improve before unrelated cord blood transplants can be recommended. Related cord blood transplants are characterized by a significantly longer time to engraftment for neutrophils and platelets. In one study with a median follow-up time of 70 months, disease-free survival at 6 years was reported to be 90%. No grade IV GVHD or extensive chronic GVHD was seen, and the cumulative incidence of primary graft failure was low (9%). However, a limitation of this treatment modality is the inability to transplant large individuals or adults using cord blood as a source of donor cells because of insufficient numbers of nucleated or stem cells in the aliquots to be transplanted and the slower engraftment of neutrophils and delays in immune reconstitution that may place the patient at increased risk of viral illness. Haploidentical transplants have been tried as well but have been reported to have a high rate of graft failure (43%). To improve on this rate of engraftment failure, patients have been treated with cyclophosphamide posttransplantation. Graft failure after one trial was still 43%, but no serious toxicities were seen. Overall, the use of alternative donors (mismatched related or unrelated) has not resulted in the same measure of success. Graft failure rates of 38%-43% have been recorded, and long-term complications have included declines in renal, pulmonary, and cardiac function because of the transplantation procedure itself. In summary, transplantation is the optimal treatment for sickle cell disease, being the only curative approach. However, clarification is needed on who is an optimal candidate, and donor sources must be expanded to balance the lesser availability of donors among minorities. Also, a clear relationship must be established between transplantation outcomes and improved quality of life, a relationship that to date has not been seen consistently or definitively. With regard to quality of life determinations, significant improvement may occur 1 year from a successful transplant, but the data are inconclusive. The reluctance of primary providers to refer individuals for transplantation is a challenge to overcome as well because, as suggested in a retrospective study of hydroxyurea, patients treated with hydroxyurea may have had better survival than those treated with allogeneic stem cell transplantation<sup>17</sup>.

### Gene Therapy

Because transplantation can be offered to relatively few individuals, hope for reaching more patients with a treatment of curative intent has focused on efforts to develop gene therapy. Recently, progress has

been speeding along toward that goal. We now know that the most common single type of genetic variation in people is the single nucleotide polymorphism (SNP). Each SNP represents a nucleotide change in the DNA genome sequence and results in unique nucleotide change(s) in the genomic sequence of DNA. As a result, unique DNA patterns for each individual are produced. Capitalizing on this knowledge, investigators from several groups demonstrated that 3 SNPs are in the BCL11A and HBB gene regions that correlate with high hemoglobin F expression. On the other hand, the gene MYB acted as a negative regulator of gamma globin expression. MYB was subsequently silenced by miR16 (microRNA R16) through binding of a 3'-untranslated region. Transfection of miR16 by Pounds and coinvestigators into human basophilic leukemia cell line KU812 cells in vitro resulted in gamma globin activation in a dose-dependent manner. This work eventuated in genetic correction of the sickle cell mutation in human cells and ultimately in actual individuals. Genome editing systems, such as transcription activator-like effector nucleases (TALENs), zinc finger nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) have been developed that can target DNA sequences around sickle mutations in the beta globin gene. These mutations are then cleaved in a site-specific manner, employing homologous donor templates to modify or replace altered DNA with the properly sequenced DNA. Gene modification of only 18% was sufficient to correct the sickle mutation and allow production of wild-type hemoglobin. On average, these efforts resulted in production of hemoglobin A, comprising 7.3% of total hemoglobin, with rates as high as 12.6%. Effort has also been made to modify the gamma globin gene because fetal hemoglobin is a more potent antisickling hemoglobin than adult or A hemoglobin. Gene therapy has progressed to the point of human trial and was reported in 2017 in a patient having sickle cell disease. Employing a lentiviral vector encoding the human HBB variant  $\beta$ A-T87Q, researchers performed ex vivo gene transfer into the patient's own hematopoietic stem cells and then performed an autologous transplant utilizing these cells. The patient had undergone myeloablation with intravenous busulfan. After transduction of CD34+ cells, a steady rise in hemoglobin AT87Q production was noted over time. The patient, previously transfusion-dependent, was able to discontinue red cell transfusions by day 88 posttransplant. The hemoglobin remained stable at levels of 10-12 grams% 6 months later. The hemoglobin percentage remained at 48% by posttransplant month 15, with a corresponding decrease in hemoglobin S levels. Despite concerns about offtarget activity of CRISPR/Cas9 or similar nuclease or vector insertional error, no adverse effects were related to the lentiviral transduction of the stem cells, perhaps because lentiviruses tend to insert themselves randomly with a bias toward integration into areas of already expressed genes, thereby minimizing transactivation of nearby genes. This property acts to tamp down the potential for insertional oncogenesis. The patient had no replication-competent lentivirus extant. Most significantly, the patient had no sickle cell-related hospitalizations or other complications. Erythropoiesis progressively showed signs of normalization. No tendency towards clonal domination was detected. This case provides optimism that we are finally moving forward in the search for other curative therapies that can be offered to a wider array of patients than has ever been possible in the past<sup>18-20</sup>.

## Conclusion

Since the first description of sickle cell disease in 1910 and discovery of its genetic origin in 1945, there has been significant progress in understanding the complex pathophysiology, pathobiology, clinical manifestation, educational, psychological effects and complication of this disease. Until recently,



however, progress in the treatment of sickle cell disease has been limited to addressing and prevention of its acute events and complications. With significant advances made in gene therapy, future treatment of sickle cell disease may be a genetic alteration which may permanently resolve the disease and prevent the significant known complications and long-term social, psychological, disabilities and mortality of this genetic disorder.

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