Sickle Cell Disease

Author: MA Bender, MD, PhD

Summary

Disease characteristics. Sickle cell disease (SCD) is characterized by variable degrees of hemolysis and intermittent episodes of vascular occlusion resulting in tissue ischemia and acute and chronic organ dysfunction. Pain and/or swelling of the hands or feet are often the earliest manifestations of sickle cell disease and usually occur in infants and young children. Consequences of hemolysis include chronic anemia, jaundice, predisposition to aplastic crisis, cholelithiasis, and delayed growth and sexual maturation. Vascular occlusion and tissue ischemia can result in acute and chronic injury to virtually every organ of the body, most significantly the spleen, brain, lungs, and kidneys.

Diagnosis/testing. The term **sickle cell disease** encompasses a group of symptomatic disorders associated with mutations in the HBB gene and defined by the presence of hemoglobin S (Hb S). Sickle cell anemia (Hb SS) accounts for 60-70% of sickle cell disease in the United States. The other forms of sickle cell disease result from co-inheritance of Hb S with other abnormal globin beta chain variants, the most common forms being sickle-hemoglobin C disease (Hb SC) and two types of sickle β-thalassemia (Hb Sβ$^+$-thalassemia and Hb Sβ^0-thalassemia). Other globin beta chain variants such as D-Punjab and O-Arab also result in sickle cell disease when co-inherited with Hb S. The diagnosis of sickle cell disease is established by demonstrating the presence of significant quantities of Hb S by high-performance liquid chromatography (HPLC), isoelectric focusing (IEF), or less commonly, hemoglobin electrophoresis. Mutation analysis for the mutations of HBB associated with hemoglobin S, hemoglobin C, hemoglobin D, hemoglobin O-Arab, β-thalassemia mutations, and other mutations associated with other specific hemoglobin variants is available on a clinical basis. HBB sequence analysis may be used following mutation analysis if it is uninformative or as the primary test to detect mutations associated with -thalassemia hemoglobin variants. Sequence analysis is available on a clinical basis.

Management. Treatment for sickle cell disease includes oral hydration and oral analgesics including opiates, acetaminophen, and ibuprofen for uncomplicated episodes of vaso-occlusive pain; severe episodes of pain require administration of parenteral analgesics. Oxygen, analgesics, antibiotics, and transfusions are used to treat acute chest syndrome. Affected individuals with fever are given broad-spectrum antibiotics such as ceftriaxone, and a macrolide antibiotic is added if acute chest syndrome (ACS) is a concern. ACS is additionally treated with oxygen, incentive spirometry, analgesia, and potentially, transfusion. For pulmonary hypertension, therapy to stop progression (exchange transfusions, oxygen therapy, hydroxyurea) is used, followed by hypertension-specific investigational agents (e.g., sidenafil) if progression continues. Monitoring of hematocrit, reticulocyte count, and cardiovascular status, and possibly transfusion, are required for aplastic crisis. Emergency transfusion is indicated when cardiovascular instability is present during severe episodes of splenic sequestration; individuals who experience multiple or severe episodes of splenic sequestration may require splenectomy. Acute treatment of children with stroke includes monitoring neurologic status and aggressive treatment of increased intracranial pressure and seizures; transfusion is generally indicated to decrease Hb S percentage to less than 30% of total hemoglobin and is generally followed by a chronic transfusion program. Chronic red blood cell transfusion therapy may prevent stroke in individuals with abnormal transcranial Doppler. Severe priapism requires hydration and analgesia and may require aspiration and irrigation. Prevention of manifestations requires hydration, avoiding climate extremes, medical interventions for early signs and symptoms, and prophylactic medications and immunizations. Individuals treated with hydroxyurea have fewer acute painful episodes, fewer episodes of acute chest syndrome, decreased need for transfusion, and improved survival. Surveillance is tailored to the specific individual and

includes yearly cbc and reticulocyte count, assessment of iron status, liver function tests, BUN, serum concentration of creatinine, and urinalysis; transcranial Doppler for individuals with Hb SS and Hb Sβ°-thalassemia starting at age two to three years; chest x-ray, pulmonary function tests, and abdominal ultrasound for individuals over age seven years; and echocardiogram for older individuals or those of any age with cardiac or pulmonary concerns. Testing of at-risk family members ensures that early diagnosis and intervention are possible before symptoms are present.

Genetic counseling. Sickle cell disease is inherited in an autosomal recessive manner. If both parents are carriers of an HBB gene mutation at conception, the sibs of an affected individual have a 25% chance of being affected, a 50% chance of being unaffected and a carrier, and a 25% chance of being unaffected and not a carrier. Once an at-risk sib is known to be unaffected, the risk of his/her being a carrier is 2/3. Carrier detection for common forms of sickle cell disease is most commonly accomplished by HPLC. Prenatal diagnosis for pregnancies at increased risk is available.

Diagnosis

Clinical Diagnosis

The term **sickle cell disease** (SCD) encompasses a group of symptomatic disorders defined by the presence of hemoglobin S (Hb S).

- **Sickle cell anemia (Hb SS)** accounts for 60-70% of sickle cell disease in the United States.
- The other forms of sickle cell disease result from co-inheritance of Hb S with other abnormal globin beta chain variants, the most common forms being **sickle-hemoglobin C disease (Hb SC)** and two types of **sickle β-thalassemia (Hb S β⁺-thalassemia and Hb S β° -thalassemia)**.
- Other globin beta chain variants such as D-Punjab and O-Arab also result in sickle cell disease when co-inherited with Hb S.

Most individuals with sickle cell disease are healthy at birth and become symptomatic later in infancy or childhood after fetal hemoglobin (Hb F) levels decrease and hemoglobin S (Hb S) levels increase. The diagnosis of sickle cell disease is suspected in infants or young children with painful swelling of the hands and feet (dactylitis or "hand-foot syndrome"), pallor, jaundice, pneumococcal sepsis or meningitis, severe anemia with splenic enlargement, or acute chest syndrome.

Testing

Hematologic Testing

Table 1 summarizes the relative quantity of hemoglobins observed by six weeks of age and typical hematologic studies by two years of age for the four most common sickle cell diseases.

Table 1. Sickle Cell Disease: Diagnostic Test Results

Disorder	Hemoglobin Separation by Age Six Weeks [1]	Phenotype	Hematologic Studies by Age Two Years				Beta Globin Genotype
			MCV [2]	Hb A_2 (%) [3]	Hb F (%)	Hb F Distribution	
SCD—SS	FS	Hemolysis and anemia by 6-12 months	N or ↑ [4]	<3.6% [4]	<25%	Heterocellular	$\beta^S \beta^S$
SCD—S β^0-thal			↓	>3.6% [4]			$\beta^0 \beta^S$
SCD—S β^+-thal	FSA	Mild or no anemia by 2 years	N or ↓	>3.6%		NA [5]	$\beta^A \beta^S$
SCD—SC	FSC			NA [6]	<15%		$\beta^S \beta^C$

Table shows typical results; exceptions occur. Some rare genotypes (e.g., SD, SO^{Arab}, SC^{Harlem}, Hb Lepore, SE) are not included.
SCD = sickle cell disease
thal = thalassemia
N = normal
↑= increased
↓= decreased
1. Hemoglobins reported in order of quantity (e.g., FSA = F>S>A)
2. Normal MCV: ≥70 at 6-12 months, ≥72 at 1-2 years
3. Hb A_2 results vary somewhat depending on laboratory methodology.
4. Hb SS with co-existent α-thalassemia may cause ↓MCV and Hb A_2 >3.6%; however, the newborn screening sample from such infants may show Hb Bart's.
5. NA = not applicable; test not indicated
6. NA = not applicable; accurate quantification requires HPLC

The diagnosis of sickle cell disease is established by demonstrating the presence of significant quantities of Hb S (by high-performance liquid chromatography, isoelectric focusing, or less commonly, cellulose acetate or citrate agar electrophoresis) and the lack of a normal globin gene (see Molecular Genetic Testing). A cbc and measure of iron status (e.g., zinc-protoporphrin) help distinguish between specific diagnostic entities.

- **High-performance liquid chromatography (HPLC)**
  - Readily separates o proteins that cannot be resolved by other means
  - Allows for accurate quantification of normal and variant hemoglobins even at low concentrations, enabling differentiation of Hb S -thalassemia from sickle cell trait (Hb AS) as well as identification of compound heterozygous disorders such as Hb S-HPFH (hereditary persistence of fetal hemoglobin) and Hb Sβ^0-thalassemia
  - Does not allow identification of Hb S-β^0-thalassemia, which requires hemoglobin electrophoresis
- **Isoelectric focusing (IEF)**
  - Capable of much higher resolution than hemoglobin electrophoresis
  - Capillary isoelectric focusing technology allows for separation of very small samples, quantification, and automation of sampling.
- **Cellulose acetate and citrate agar electrophoresis**
  - o Useful for quick screening of a small number of samples
  - o Sample bands are relatively wide and many abnormal hemoglobins overlap.

- Quantitative densitometry of abnormal hemoglobins is inaccurate at low concentrations (i.e., Hb A_2, Hb F).
- Is being supplanted by HPLC

Peripheral blood smear

- Sickle cells, nucleated red blood cells, and target cells may be seen. Other abnormal forms may be present depending on the specific genotype.
- Presence of Howell-Jolly bodies indicates hyposplenism.
- Neutrophil and platelet numbers are often increased.

Kleihauer-Betke test. This acid-elution test detects the presence of cells with a high fetal hemoglobin content and can be used to characterize co-existent HPFH with sickle cell disease.

The solubility test (i.e., sickledex, sickleprep, or sicklequick) utilizes the relative insolubility of deoxygenated Hb S in solutions of high molarity. Hemolysates containing Hb S precipitate in the test solution while those without Hb S remain in solution. The solubility test has no place in the diagnosis of sickle cell disease because (1) it does not differentiate sickle cell disease from sickle cell trait (Hb AS); (2) high levels of Hb F may cause false negative results in neonates with sickle cell disease; and (3) it may miss some clinically significant forms of sickle hemoglobinopathies (e.g., Hb S/C).

Newborn screening. Because of the high morbidity and mortality of sickle cell disease in undiagnosed toddlers, all 50 states, the District of Columbia, Puerto Rico, and the Virgin Islands currently provide universal newborn screening for sickle cell disease. The vast majority of new cases are diagnosed at birth.

Note: New Hampshire has approved, but not yet implemented, newborn screening [National Newborn Screening Status Report (pdf)].

The majority of newborn screening programs perform isoelectric focusing of an elute of the dried blood spots obtained for screening [Kleman et al 1989 , Steinberg 1991 , Shafer et al 1996]. A few programs use HPLC or cellulose acetate electrophoresis as the initial screening method.

Hemoglobins identified by newborn screening are generally reported in order of quantity. For example, more fetal hemoglobin (Hb F) than adult hemoglobin (Hb A) is present at birth; thus, most infants show Hb FA on newborn screening.

Specimens with abnormal screening results are retested using a second, complementary electrophoretic technique, HPLC, immunologic tests, or DNA-based assay [Steinberg 1991].

Infants with hemoglobins that suggest sickle cell disease or other clinically significant hemoglobinopathies (Table 1) require confirmatory testing of a separate blood sample by six weeks of age.

Molecular Genetic Testing

Gene. The term sickle cell disease encompasses a group of symptomatic disorders associated with mutations in the HBB gene and defined by the presence of hemoglobin S (Hb S; E6V mutation).

- Sickle cell anemia (also known as homozygous sickle cell disease and Hb SS) used to account for 60-70% of sickle cell disease in the United States; however, because of increasing numbers of births with mixed ethnic background, this number is falling.
- Sickle cell disease may also result from co-inheritance of the E6V hemoglobin S mutation with an *HBB* mutation associated with another abnormal hemoglobin variants including:

- Hemoglobin C (Hb C; E6K mutation): sickle-hemoglobin C disease (Hb SC)
- Beta-thalassemia mutations: Sβ^+-thalassemia and Sβ^0-thalassemia
- Hemoglobin D (D-Punjab; E121Q mutation)
- Hemoglobin O (O-Arab; E121K mutation)

Molecular genetic testing: Clinical uses

- Confirmatory testing
- Carrier testing
- Prenatal diagnosis

Molecular genetic testing: Clinical methods

- **Targeted mutation analysis.** Testing for the E6V mutation of HBB associated with hemoglobin S, the E6K mutation associated with hemoglobin C, the E121Q mutation associated with hemoglobin D, the E121K mutation associated with hemoglobin O-Arab, -thalassemia mutations, and other mutations associated with other specific hemoglobin variants is available on a clinical basis. The Hb S mutation destroys the recognition sites for the restriction enzymes Mni I, Dde I, Mst II, and others, making it easily detectable by restriction fragment length polymorphism (RFLP) analysis. Increasingly, a variety of PCR-based techniques are being used to identify the Hb S mutation.
- **Sequence analysis.** HBB sequence analysis may be used following targeted mutation analysis if it is uninformative or as the primary test to detect mutations associated with β-thalassemia hemoglobin variants.

Table 2 summarizes molecular genetic testing for this disorder.

Table 2. Molecular Genetic Testing for Carrier Testing and Prenatal Diagnosis in Sickle Cell Disease

Test Method	Mutations Detected [1]		Mutation Detection Rate in Individuals Known to be Carriers from Hematologic Studies
Targeted mutation analysis	Hemoglobin S (E6V)	Hemoglobin C (E6K)	100%
		Hemoglobin D (E121Q)	
		Hemoglobin O (E121K)	
Sequence analysis		*HBB* mutations	~99% [2]

1. Targeted mutation analysis for additional HBB mutations is available on a clinical basis.
2. As reported by laboratories in the GeneTests Laboratory Directory

Testing Strategy for a Proband

The multiple testing strategies possible vary depending on the specific diagnosis, proband's age, family history, and availability of parents for testing.

Newborns. When screening with IEF or HPLC detects a clinically significant hemoglobinopathy, the result should be confirmed within six weeks with either of these assays or DNA testing.

- For compound heterozygotes (e.g., Hb SC, SD, or SO) a repeat test is adequate.
- Newborns with Hb F>Hb S could have homozygous sickle cell (Hb SS), Sβ°-thalassemia, or Sβ+-thalassemia with a low level of Hb A. These hemoglobinopathies can be difficult to distinguish in the newborn period when 95% of hemoglobin is Hb F. Further testing for these infants, as well as newborns diagnosed with Sβ+-thalassemia can include molecular testing and/or hematologic testing of parents as described above.

Infants about one year of age. Regardless of the outcome of testing in the newborn period, additional testing that should be done at about one year of age (once Hb F levels have fallen) includes a cbc, reticulocyte count, some form of electrophoresis or HPLC, a measure of iron status, and inclusion body preparation. This helps assess co-existing alpha-thalassemia and increases detection of Sβ-thalassemia syndromes. This is important for genetic counseling and for providing insight into disease-specific outcomes.

Individuals over age one year. A one-time assessment with a cbc, reticulocyte count, some form of electrophoresis or HPLC, a measure of iron status, and inclusion body preparation are indicated.

Genetically Related (Allelic) Disorders

- Beta-thalassemia is caused by mutations in HBB that result in decreased or absent production of beta globin (hemoglobin A). More than 200 different mutations have been described.

Clinical Description

Natural History

The clinical manifestations of sickle cell disease result from variable degrees of hemolysis and intermittent episodes of vascular occlusion leading to tissue ischemia and acute and chronic organ dysfunction. Delays in growth and sexual maturation may result. Consequences of hemolysis include chronic anemia, jaundice, predisposition to aplastic crisis, and cholelithiasis. Vascular occlusion and tissue ischemia can result in acute and chronic injury to virtually every organ of the body, most significantly the spleen, brain, lungs, kidneys, and penis. [Vichinsky 1991 , 2002].

Dactylitis. Dactylitis, the term for pain and/or swelling of the hands or feet, is often the earliest manifestation of SCD. The dorsa of the extremities are most often involved; one or all four extremities can be involved. When present, dactylitis usually occurs in infants and children. Although immediate sequelae are rare, dactylitis is a risk factor for future complications [Miller et al 2000].

Vaso-occlusive pain crisis. Pain is the most frequent cause of recurrent morbidity in sickle cell disease and accounts for the majority of sickle cell disease-related hospital admissions as well as school and work absences. Pain results from vaso-occlusion and ischemic tissue damage. Young children more often complain of pain in their extremities, whereas older individuals more commonly experience pain in the head, chest, abdomen, and back.

Acute chest syndrome (ACS). Different centers use differing definitions of acute chest syndrome but they all include the presence of a new infiltrate identified on a chest radiograph in a person with a sickle hemoglobinopathy. At some centers this must be accompanied or preceded by lower respiratory tract symptoms and/or hypoxemia, and/or fever. ACS may present as an acute respiratory illness or may develop after two to three days of severe vaso-occlusive pain. ACS can be a complication of general anesthesia. A high index of suspicion is need as the presenting signs and symptoms of ACS can be highly variable and affected individuals may have a normal physical examination.

Pulmonary hypertension. While it has long been known that individuals with SCD are at risk for developing pulmonary hypertension, awareness of the high prevalence is increasing. Some individuals with pulmonary hypertension may develop exercise intolerance or other cardiac or respiratory complaints; others are relatively asymptomatic in the early stages.

Aplastic crisis. Aplastic crisis is characterized by an exacerbation of the individual's baseline anemia with a substantially decreased reticulocyte count, typically less than 1%. Most aplastic crises are caused by acute infection with human parvovirus B19, resulting in transient red cell aplasia.

Splenic sequestration. Splenic sequestration is characterized by an acutely enlarging spleen with hemoglobin more than 2g/dL below the affected individual's baseline value. Mild-to-moderate thrombocytopenia may also be present. Splenic sequestration occurs in 10-30% of children with sickle cell disease, most commonly between six months and three years of age. Individuals may experience abdominal pain, nausea, and vomiting. Splenic sequestration may follow a febrile illness. Severe splenic sequestration may progress rapidly to shock and death.

Stroke. Strokes are among the most catastrophic manifestations of sickle cell disease. Common presenting signs and symptoms include severe headache, hemiparesis, monoparesis, seizures, aphasia or dysphasia, cranial nerve palsies, or mental status changes. Overt strokes occur in as many as 11% of children with sickle cell disease with the peak occurrence between the ages of two and nine years. An additional peak is observed in older individuals.

An additional 17% of individuals with SSD develop "silent infarcts" with neurocognitive changes without a recognized acute event. These persons may be at increased risk for additional silent and overt infarcts. Thus, a "silent infarct" should not be thought of as a clinically insignificant condition.

Infection. Individuals with sickle cell disease develop splenic dysfunction as early as three months of age; thus, young children with sickle cell disease are at high risk for septicemia and meningitis with pneumococci and other encapsulated bacteria. The single most common cause of death in children with sickle cell disease is *Streptococcus pneumonia* sepsis, with the risk of death being highest in the first three years of life. Individuals with sickle cell disease are also at increased risk of infections such as osteomyelitis caused by *Staphylococcus areus* or other organisms such as *Salmonella* species.

Priapism. Painful unwanted erections commonly occur in males sickle cell disease, often starting during the early morning hours. Individuals may have intermittent episodes of priapism lasting fewer than two to four hours that are often recurrent and may precede a more severe episode. Severe episodes lasting more than two to four hours need rapid intervention as prolonged priapism may result in permanent tissue damage and impotence.

Other. Other complications of sickle cell disease include avascular necrosis of the femoral head, nephropathy, restrictive lung disease, cholelithiasis, retinopathy, cardiomyopathy, delayed growth and sexual maturation, and leg ulcers.

Heterozygotes. Heterozygotes for Hb S have hemoglobin AS (Hb AS) (also called **sickle cell trait**). They usually have no symptoms. Heterozygous individuals are not anemic and have normal red cell indices. Although heterozygotes express Hb S, the amount of Hb S present is insufficient to produce sickling manifestations under normal circumstances. Some heterozygotes may have abnormal laboratory findings, such as micro-hematuria.

Genotype-Phenotype Correlations

Although a tremendous amount of individual variability occurs, individuals with Hb SS and Sβ°-thalassemia are generally more severely affected than individuals with Hb SC or Sβ+-thalassemia.

In individuals with Hb SC:

- Longer red cell life span and higher hemoglobin concentration tend to result in fewer vaso-occlusive pain crises and fewer aplastic crises.
- Splenomegaly and the accompanying risk of splenic sequestration can persist well beyond early childhood.
- Proliferative retinopathy and avascular necrosis are more likely to develop than in those with other sickle hemoglobinopathies.

Nomenclature

Historically in the United States, the term "sickle cell anemia" was in common usage to describe homozygosity for Hb SS. With increased awareness of the broad spectrum of clinically significant sickle hemoglobinopathies with varying degrees of anemia, the trend has been to use the umbrella term "sickle cell disease." The term sickle cell disease should be followed by a detailed genotypic description for the individual (e.g., Hb SS, Hb SC, or sickle beta-zero thalassemia).

Prevalence

HBB alleles associated with sickle cell disease are common in persons of African, Mediterranean, Middle Eastern, and Indian ancestry and in persons from the Caribbean and parts of Central and South America, but can be found in individuals of any ethnic background.

Among African-Americans, the prevalence of sickle cell trait (Hb AS) is 8-10%, resulting in the birth of approximately 2000 infants with sickle cell anemia (Hb SS) annually in the US. Approximately one in every 250-600 African-Americans born in the US have sickle cell disease [Vichinsky et al 1988 , Shafer et al 1996].

The prevalence of *HBB* alleles associated with sickle cell disease is even higher in other parts of the world — as high as 25-30% in West Africa, resulting in an estimated annual birth of 120,000 babies with sickle cell disease in Africa.

Differential Diagnosis

Once the presence of Hb S has been confirmed, the differential diagnosis is between clinically significant, less significant, and carrier states.

Clinically significant

- Homozygous S/S (i.e., Hb SS)
- Compound heterozygotes including:
    - Hb SC, Hb SD, Hb SO-Arab
    - Hb Sβ°-thalassemia
    - Some forms of Hb Sβ+-thalassemia

Less clinically significant

- Some forms of Hb Sβ+-thalassemia
- Hb SE

Management

Evaluations at Initial Diagnosis to Establish the Extent of Disease

Initial evaluations vary with the age and clinical status of the individual:

- **Newborns.** Confirmation of diagnosis
- **Infants** (age 9-12 months, usually when fetal hemoglobin levels have fallen). Reticulocyte count, cbc, measurement of Hb F (%), and assessment of iron status
- **Older individuals.** See Surveillance.

Treatment of Manifestations

Lifelong comprehensive care is necessary to minimize morbidity, to reduce early mortality, and to maximize quality of life.

Education of parents, caregivers, and affected individuals is essential.

- Families must appreciate the importance of routine health maintenance visits and prophylactic medications.
- Warning signs of acute illness such as fever, respiratory symptoms, pallor, lethargy, splenic enlargement, and neurologic changes must be reviewed regularly.
- All families should have a plan in place for 24-hour access to a medical facility that can provide urgent evaluation and treatment of acute illnesses such as fever, acute chest syndrome, and splenic sequestration.
- A plan to deal with mild-to-moderate episodes of pain should be in place.
- Families should be provided baseline (steady state) laboratory values for purposes of comparison, as values often change during acute illness [NHLBI 2002].

General management of specific problems is discussed below [Vichinsky 1991 , Charache et al 1995 , Styles & Vichinsky 1996 , Gladwin & Rodgers 2000 , Walters et al 2000]:

- **Vaso-occlusive pain crisis including dactylitis.** Many uncomplicated episodes of pain can be managed at home with oral hydration and oral analgesics including opiates, acetaminophen, and ibuprofen.

  More severe episodes of pain require hospitalization and administration of parenteral analgesics.

  - Optimal analgesia is generally achieved by morphine, or other opiate, given around the clock or by patient-controlled analgesia.
  - Nonsteroidal anti-inflammatory drugs (NSAIDS) such as ketorolac and/or acetaminophen may be used to augment the analgesic effect of opiates.
  - Adequate but not excessive hydration should be maintained and individuals should be monitored closely for the development of other complications such as acute chest syndrome or splenic sequestration.
- **Infection/fever.** All affected individuals with temperature greater than 38.5° C require rapid triage and physical assessment, urgent cbc and reticulocyte count, blood culture (other cultures should be obtained as clinically indicated), and chest x-ray.

  Individuals with fever should be given broad-spectrum empiric antibiotics such as ceftriaxone pending culture results.

    - A macrolide antibiotic should be added if ACS is a concern.
    - Additional antibiotics should be added only for proven or suspected meningitis or other severe illness.
- **Acute chest syndrome (ACS).** The index of suspicion for acute chest syndrome should be high when individuals with SCD have fever, chest pain, or respiratory symptoms.
    - Because physical signs are variable (and can be absent), the threshold for obtaining a chest X-ray should be low.
    - Those suspected of having acute chest syndrome should be aggressively treated with oxygen, analgesics, and antibiotics (including a macrolide).
    - Simple transfusion or exchange transfusion may be necessary.
- **Aplastic crisis.** Monitoring of hematocrit (both absolute and compared with the individual's baseline), reticulocyte count, and cardiovascular status are required. Transfusion may be necessary.
- **Splenic sequestration.** Severe episodes of splenic sequestration may progress rapidly to cardiovascular collapse and death; thus, emergency transfusion is indicated when signs of cardiovascular instability are present.

  Individuals who experience multiple or severe episodes of splenic sequestration may require splenectomy.

  Pulmonary hypertension. Although no consensus regarding the optimal management for pulmonary hypertension exists, a two-step approach is reasonable:

    1. Optimization of sickle cell disease-related therapy to stop progression (e.g., exchange transfusions, oxygen therapy, hydroxyurea)
    2. If pulmonary hypertension continues to progress or is severe at diagnosis, use of pulmonary hypertension-specific investigational agents (e.g., sidenafil)
- **Stroke.** Any history of a neurologic event or acute neurologic symptom warrants prompt evaluation including a cbc with reticulocyte count and a non-contrast CT scan or MRI.

  Treatment for children with acute stroke:

    - Monitoring neurologic status as well as aggressive treatment of increased intracranial pressure and seizures if present
    - Exchange transfusion with the goal of decreasing Hb S percentage to less than 30% of the total hemoglobin

  Without preventive therapy, as many as 60-90% of individuals who have had a stroke will have a second stroke within three years. Thus, in most cases, a preventive chronic transfusion protocol is initiated after a CNS event (see Prevention of Primary Manifestations).

  No consensus regarding the management of individuals with silent infarcts exists. A multi-center trial comparing observation versus a chronic transfusion regimen is underway.

- **Priapism.** Episodes of severe priapism require urgent evaluation and treatment including hydration and analgesia, and may require aspiration and irrigation by a urologist.

Prevention of Primary Manifestations

Ongoing education for all individuals with SCD is essential to help minimize morbidity and mortality. Education includes a regular review of interventions such as:

- Maintaining hydration and avoiding extremes of climate
- Monitoring for signs and symptoms requiring acute medical intervention

- Early detection of chronic complications
- Updates on new therapies

Disease-modulating therapies are reviewed by Vichinsky (2002).

Chronic red blood cell transfusion therapy. The initial goal of chronic red blood cell transfusion therapy is to maintain the percentage of Hb S less than 30%.

Chronic red blood cell transfusion therapy may be warranted for:

- Primary prevention of stroke in individuals with an abnormal transcranial Doppler
- Prevention of stroke recurrence
- Treatment of chronic debilitating pain and pulmonary hypertension

Complications of chronic red blood cell transfusion therapy include iron overload, allo-immunization, and infection.

Hydroxyurea. Hydroxyurea, the most prescribed therapy for sickle cell disease, may benefit individuals with SCD via several mechanisms:

- Induction of Hb F synthesis resulting in decreased sickling and improved red-cell survival
- Lowering the white blood cell (WBC) count
- Metabolization into nitric oxide, a potent vasodilator

Individuals treated with hydroxyurea have significantly fewer acute painful episodes, fewer episodes of acute chest syndrome, decreased need for transfusion, and most importantly, improved survival [Steinberg et al 2003]. However, hydroxyurea does not appear to prevent the cerebrovascular complications of sickle cell disease.

Individuals treated with hydroxyurea must be monitored closely for significant myelosuppression.

Stem cell transplantation. Stem cell transplantation from a normal donor or one with sickle cell trait can be curative in individuals with sickle cell disease, but the risks and morbidity associated with this procedure have limited its use to a select group of individuals with significant complications who have a matched sibling stem-cell donor [Walters et al 2000]. Among these individuals, more than 90% survive and approximately 85% survive free from sickle cell disease.

New developments in stem cell transplantation such as non-myeloablative regimens, better immunosuppression, and alternative sources of stem cells are making stem cell transplantation an option for an increasing number of individuals with sickle cell disease.

Because the criteria, risks, and benefits of transplantation are changing rapidly, it is important for families and providers to discuss the risks and benefits with a transplantation center.

Prevention of Secondary Complications

Penicillin prophylaxis prevents 80% of life-threatening episodes of childhood *Streptococcus pneumoniae sepsis* [Gaston et al 1986].

- By two months of age, all infants with sickle cell disease should receive penicillin V potassium prophylaxis, 125 mg orally, twice a day.
- At age three years, the dose is increased to 250 mg orally, twice a day, and continued until at least age five years.

Erythromycin prophylaxis is an alternative for individuals allergic to penicillin.

Folic acid supplementation may also be considered.

Immunizations. Timely administration of the following vaccines is essential:

- *Hemophilus influenzae* type b (Hib) vaccine
- 7-valent pneumococcal conjugate vaccine
- 23-valent pneumococcal polysaccharide vaccine

Additional prophylactic measures:

- Quadrivalent meningococcal polysaccharide vaccine should be considered.
- Annual influenza immunization is recommended.

Surveillance

Surveillance should be tailored to a specific individual's clinical history; however, most individuals benefit from routine age-dependent screening to allow early detection and treatment of end-organ damage. The following are general guidelines:

- **Routine**
  - Developmental and/or neurocognitive assessments
  - Social work asssessments with emphasis on support, resources, and impact of the disease on lifestyle
  - Nutritional and dental evaluations
- **Yearly.** A cbc and reticulocyte count, assessment of iron status, liver function tests (LFTs), BUN, serum concentration of creatinine (Cr), and urinalysis (UA)
- **Yearly starting at age two to three years.** Transcranial Doppler for all individuals with Hb SS and Hb Sβ^0-thalassemia (as well as some others) by someone certified to record velocity of arterial blood flow for comparsion to national studies to determine the risk of stroke. Individuals with an abnormally high velocity of arterial blood flow have a high rate of stroke, which can be prevented by chronic red blood cell transfusion therapy. Initial studies suggest that this approach is decreasing the incidence of overt stroke in individuals with SCD.
- **Yearly for individuals over age seven years.** Chest X-ray, pulmonary function tests (PFTs), and abdominal US
- **For older individuals or individuals of any age with cardiac or pulmonary concerns.** Echocardiogram to determine the tricuspid regurgitation (TR) jet. Guidelines for initiation and frequency of screening have not been established.

Additional studies should be tailored to the affected individual's clinical history.

Agents/Circumstances to Avoid

Education for individuals with SCD involves learning how to control one's environment to minimize the chance of exacerbations. Environmental controls include avoiding the following:

- Dehydration
- Extremes of temperature (e.g., swimming in cold water can trigger a pain episode)
- Physical exhaustion
- Extremely high altitude without oxygen supplementation

Meperidine should be avoided as first-line therapy because of potential CNS toxicity.

Testing of Relatives at Risk

A family history should be obtained to determine those family members at risk for being carriers or having the disease (see Genetic Counseling for genetic risk assessment).

Testing of family members at risk for SCD is suggested so that early diagnosis and intervention are possible before symptoms present.

Therapies Under Investigation

Many factors in sickle-cell-induced ischemic injury are regulated by nitric oxide (NO) including blood vessel tone, leukocyte and platelet activity, and endothelial adhesion [Gladwin & Rodgers 2000 , Hebbel 2000]. Nitrous oxide concentrations are low in sickle cell disease. Pilot studies treating individuals with NO have shown beneficial effects; several studies are currently underway.

L-arginine, a precursor of NO, is depleted in individuals with SCD and is being intensively studied as well.

Other novel agents currently being investigated as potential therapies for sickle cell disease include modulators of the transport systems involved in cellular dehydration, modifiers of endothelial adherence, antithrombotic therapy, and poloxamer 188, a non-ionic surfactant copolymer that improves microvascular blood flow [Orringer et al 2001]. Most recently, the use of magnesium and the Gardos channel inhibitor ICA-17043 as agents to prevent cellular dehydration is being investigated.

Other drugs that increase the percentage of Hb F such as short chain fatty acids (i.e., butyrate or valproic acid) and 5-azacytidine are currently being investigated as possible therapies for sickle cell disease [Hagar & Vichinsky 2000 , Atweh & Loukopoulos 2001]. Increasing analogues of these drugs with higher therapeutic ratios are being evaluated (e.g., 5-aza-2'-deoxycytidine).

Gene therapy. The importance of a single nucleotide substitution in the pathogenesis of sickle cell disease makes the presence of the sickle cell allele an ideal candidate for gene therapy. Ideally gene therapy would lead to an increase in non-sickle beta-like chains, while lowering the number of sickle chains.

While many approaches could increase the synthesis of non-sickle chains (e.g., using transactivators to stimulate the minimally expressed delta gene or the fetal or embryonic genes), the primary focus has been to add a normal beta- like gene, potentially modified to have additional anti-sickling properties.

Other promising approaches are induction of embryonic alpha-like chains that, when forming tetramers with sickle chains, are less likely to polymerize, or addition of a vector to inhibit production of the sickle globin.

Combined approaches, simultaneously expressing a normal beta-chain while inhibiting sickling, have shown promise.

Successful expression of the human beta globin gene by retroviral vectors in a mouse model for sickle cell anemia has demonstrated the potential of these approaches. The efficiency of gene transfer is still a hurdle, but strategies for in vivo selection may be useful. The safety of these approaches in human subjects has recently been called into question by the development of leukemia in individuals participating in a clinical trial of gene therapy for X-linked severe combined immunodeficiency (XLSCID).

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional.

Mode of Inheritance

Sickle cell disease is inherited in an autosomal recessive manner.

Risk to Family Members

Parents of a proband

In most instances, the parents of an individual with Hb SS are heterozygotes and therefore carry one Hb S allele.

For probands with other forms of sickle cell disease such as Hb SC or Hb S -thalassemia, each parent is usually heterozygous for a different HBB mutation.

- Heterozygotes (carriers) are asymptomatic.
- As the carrier rate for Hb S in certain populations is high, it is possible that a parent is homozygous (i.e., Hb SS) or compound heterozygous (e.g., Hb S -thalassemia) rather than heterozygous.

Sibs of a proband

- If both parents are carriers of an HBB gene mutation at conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier.
    - Once an at-risk sib is known to be unaffected, the risk of his/her being a carrier is 2/3.
- If one parent • is homozygous and the other parent is heterozygous for an HBB gene mutation, each sib of an affected individual has a 50% chance of being affected and a 50% chance of being an asymptomatic carrier.
- Once an at-risk sib is known to be unaffected, he/she can be assumed to be a carrier.
- If both parents are homozygous, all sibs of an affected individual will be affected.
- Heterozygotes (carriers) are asymptomatic.

Offspring of a proband. The offspring of an individual with sickle cell disease are obligate heterozygotes (carriers) for a disease-causing mutation in the HBB gene. If the reproductive partner of an affected individual is heterozygous for Hb S or another sickle cell disease-causing HBB mutation, each offspring will be at a 50% risk of having sickle cell disease.

Other family members of a proband. Each sib of the proband's parents is at a 50% or greater risk of being a carrier. If one sib of a proband's parent is affected, each other (unaffected) sib of the proband's parent is at a 67% (2/3) risk of being a carrier.

Carrier Detection

Carrier detection for common forms of sickle cell disease involving qualitative abnormalities (i.e., abnormal hemoglobins) is most commonly accomplished by HPLC. Note that HPLC may not

detect quantitative abnormalities such as thalassemias, which, when inherited with a Hb S allele, result in a significant hemoglobinopathy. Other methods such as IEF and DNA-based assays may also be used. Typical parental genotypes are described in Table 3.

Table 3. Sickle Cell Disease: Parent and Proband Genotypes

SCD Genotype of the Proband	Typical Parental Genotypes [1]	
	One Parent	Other Parent
SS	AS	AS
Sβ°-thal		A ↓MCV [2] ↑Hb A_2 N or ↑Hb F [2]
Sβ+-thal		
SC		AC

Table shows typical results; exceptions occur. Some rare genotypes (e.g., SD, SO^{Arab}, SC^{Harlem}, Hb Lepore, SE) are not included.
SCD = sickle cell disease
thal = thalassemia
N = normal
↑= increased
↓= decreased
1. Assumes that uniparental disomy is absent and that both parents are heterozygous. In some cases, parents may be homozygous or compound heterozygous.
2. Low MCV, high Hb A_2 or F

Related Genetic Counseling Issues

It must be kept in mind that non-sickle beta globin disorders (e.g., beta-thalassemia) can interact with a sickle cell disease mutation to cause clinically significant disease. As a result, family members with no Hb S can still have a child with a significant sickle hemoglobinopathy. For example, if one parent has sickle cell trait and the other has beta-thalassemia trait, it would be correct to state that although one parent is not a sickle cell carrier, there is still a 25% chance that each pregnancy would have a significant hemoglobinopathy.

Family planning. The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal testing is before pregnancy.

Early testing. Building community awareness of SCD in populations at high risk is an important component in facilitating early testing.

DNA banking. DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, mutations, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals.

Prenatal Testing

Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or chorionic villus sampling (CVS) at about 10-12 weeks' gestation.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Because one parent may have a non-Hb S mutation that can interact with Hb S to cause a sickle hemoglobinopathy (e.g., Hb C or Hb -thalassemia) both disease-causing HBB alleles of the carrier parents must be identified before prenatal testing can be performed. Because of the large variation in clinical course, it is not possible to accurately predict the course of sickle cell disease in an individual.

When the mother is a known carrier and the father is unknown and/or unavailable for testing, options for prenatal testing can be explored in the context of formal genetic counseling.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutations have been identified in an affected family member in a research or clinical laboratory.

Molecular Genetics

Information in the Molecular Genetics tables may differ from that in the text; tables may contain more recent information. —ED.

Molecular Genetics of Sickle Cell Disease

Gene Symbol	Chromosomal Locus	Protein Name
HBB	11p15.5	Hemoglobin subunit beta

Data are compiled from the following standard references: Gene symbol from HUGO; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from Swiss-Prot.

OMIM Entries for Sickle Cell Disease

141900	HEMOGLOBIN--BETA LOCUS; HBB
603903	SICKLE CELL ANEMIA

Genomic Databases for Sickle Cell Disease

Gene Symbol	Locus Specific	Entrez Gene	HGMD	GeneCards	GDB	GenAtlas
HBB	HBB	141900	HBB	*HBB*	119297	*HBB*

Note: HGMD requires registration.

Molecular Genetic Pathogenesis

Hemoglobin S results from the substitution of valine for glutamic acid in the second nucleotide of the sixth codon of the -globin chain. In deoxygenated sickle hemoglobin, an interaction between the 6 valine and the complementary regions on adjacent molecules can result in the formation of highly ordered molecular polymers that aggregate and distort the shape of the red blood cells, making them brittle and poorly deformable. Sickle hemoglobin is also injurious to the red cell

membrane, resulting in cellular dehydration, oxidative damage, and increased adherence to endothelial cells [Gladwin & Rodgers 2000 , Hebbel 2000 , Nagel 2001]. Other factors contributing to the pathophysiology of sickle cell include leukocytosis, resulting in increased production of injurious cytokines and altered blood flow, coagulation abnormalities, and abnormal vascular regulation. The net result of these abnormalities is shortened red cell lifespan or hemolysis and intermittent vascular occlusion and a state of chronic inflammation.

Normal allelic variants: The HBB gene, which spans 1.6 kb, contains three exons and both 5' and 3' untranslated regions. The HBB gene is regulated by an adjacent 5' promoter, which contains a TATA, CAAT, and duplicated CACCC boxes, and an upstream regulatory element dubbed the locus control region (LCR). A number of transcription factors regulate the function of the HBB gene including the erythroid Kruppel-like factor (EKLF) which binds the proximal CACCC box and whose knockout in the mouse leads to a thalassemia-like clinical picture. Many other factors are critical, but their deletion results in milder phenotypes because of compensation by other factors. The HBB gene is contained within the HBB gene cluster, which also includes the genes encoding the delta globin chain, A gamma and G gamma chains, and a pseudo HBB gene and epsilon.

Pathologic allelic variants: Hemoglobin S results from the substitution of valine for glutamic acid in the second nucleotide of the sixth codon of the β-globin chain.

Normal gene product: The *HBB* gene encodes the hemoglobin beta chain. The normal heterotetrameric protein hemoglobin A (Hb A) is made up of two hemoglobin alpha chains, two hemoglobin beta chains, and four heme moieties.

Abnormal gene product: Sickle hemoglobin (Hb S) results from a single point mutation in which the codon determining the amino acid at position β^6 has changed from GAG coding for glutamic acid to GTG coding for valine. Hb S is a heterotetrameric protein made up of two hemoglobin alpha chains, two hemoglobin sickle-beta chains, and four heme moieties.

References

Published Statements and Policies Regarding Genetic Testing

No specific guidelines regarding genetic testing for sickle cell disease have been developed.

Literature Cited

- Atweh GF and Loukopoulos D (2001) Pharmacological induction of fetal hemoglobin in sickle cell disease and beta-thalassemia. Semin Hematol 38:367-73 [Medline]

- Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, McMahon RP, Bonds DR (1995) Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med 332:1317-22 [Medline]

- Gaston MH, Verter JI, Woods G, Pegelow C, Kelleher J, Presbury G, Zarkowsky H, Vichinsky E, Iyer R, Lobel JS, et al (1986) Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. N Engl J Med 314:1593-9 [Medline]

- Gladwin MT and Rodgers GP (2000) Pathogenesis and treatment of acute chest syndrome of sickle-cell anaemia. Lancet 355:1476-8 [Medline]

- Hagar RW and Vichinsky EP (2000) Major changes in sickle cell disease. Adv Pediatr 47:249-72 [Medline]

- Hebbel RP (2000) Blockade of adhesion of sickle cells to endothelium by monoclonal antibodies. N Engl J Med 342:1910-2 [Medline]

- Kleman KM, Vichinsky E, Lubin BH (1989) Experience with newborn screening using isoelectric focusing. Pediatrics 83:852-4 [Medline]

- Miller ST, Sleeper LA, Pegelow CH, Enos LE, Wang WC, Weiner SJ, Wethers DL, Smith J, Kinney TR (2000) Prediction of adverse outcomes in children with sickle cell disease. N Engl J Med 342:83-9 [Medline]

- Nagel RL (2001) Pleiotropic and epistatic effects in sickle cell anemia. Curr Opin Hematol 8:105-10 [Medline]

- National Heart, Lung, and Blood Institute: Division of Blood Diseases and Resources (2002) The Management of Sickle Cell Disease (pdf, electronic resource), 4 ed. National Institutes of Health, Bethesda, MD

- Orringer EP, Casella JF, Ataga KI, Koshy M, Adams-Graves P, Luchtman-Jones L, Wun T, Watanabe M, Shafer F, Kutlar A, Abboud M, Steinberg M, Adler B, Swerdlow P, Terregino C, Saccente S, Files B, Ballas S, Brown R, Wojtowicz-Praga S, Grindel JM (2001) Purified poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease: A randomized controlled trial. JAMA 286:2099-106 [Medline]

- Shafer FE, Lorey F, Cunningham GC, Klumpp C, Vichinsky E, Lubin B (1996) Newborn screening for sickle cell disease: 4 years of experience from California's newborn screening program. J Pediatr Hematol Oncol 18:36-41 [Medline]

- Steinberg M (1991) Newborn screening for hemoglobinopathies: Program development and laboratory methods. Ad Hoc Newborn Screening Committee. National Institutes of Health, Bethesda, MD

- Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, Orringer E, Bellevue R, Olivieri N, Eckman J, Varma M, Ramirez G, Adler B, Smith W, Carlos T, Ataga K, DeCastro L, Bigelow C, Saunthararajah Y, Telfer M, Vichinsky E, Claster S, Shurin S, Bridges K, Waclawiw M, Bonds D, Terrin M (2003) Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA 289:1645-51 [Medline]

- Styles LA and Vichinsky EP (1996) Core decompression in avascular necrosis of the hip in sickle-cell disease. Am J Hematol 52:103-7 [Medline]

- Vichinsky E, Hurst D, Earles A, Kleman K, Lubin B (1988) Newborn screening for sickle cell disease: effect on mortality. Pediatrics 81:749-55 [Medline]

- Vichinsky EP (1991) Comprehensive care in sickle cell disease: its impact on morbidity and mortality. Semin Hematol 28:220-6 [Medline]

- Vichinsky E (2002) New therapies in sickle cell disease. Lancet 360:629-31 [Medline]

- Walters MC, Storb R, Patience M, Leisenring W, Taylor T, Sanders JE, Buchanan GE, Rogers ZR, Dinndorf P, Davies SC, Roberts IA, Dickerhoff R, Yeager AM, Hsu L, Kurtzberg J, Ohene-Frempong K, Bunin N, Bernaudin F, Wong WY, Scott JP, Margolis D, Vichinsky E, Wall DA, Wayne AS, Pegelow C, Redding-Lallinger R, Wiley J, Klemperer M, Mentzer WC, Smith FO, Sullivan KM (2000) Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation of bone marrow transplantation for sickle cell disease. Blood 95:1918-24 [Medline]

Author Information

MA Bender, MD, PhD
Department of Pediatric Hematology and Oncology
Fred Hutchinson Cancer Research Center
Seattle

This Article has been published on GeneReviews WebSite :

Bender MA; Schlis K; Vichinsky E. (Updated on: Apr 03 ; Sept 03; Last Update : March 06) **Sickle Cell Disease**. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2008. Available at http://www.genetests.org. Accessed [9-28-07].