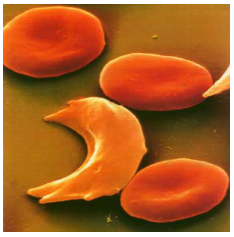
Sickle Cell Anemia



Sickle Cell Disease

Sickle Cell Anemia is the most commonly inherited blood disorder in the United States and represents one form of anemia. Symptomatically, this disorder was known for quite some time in Africa before it was recognized in the western hemisphere, with reports dating back to 1670 in Ghana (1). The first observation of these abnormal sickle-shaped cells in human blood did not occur until 1910 by James Herrick; it was this report that brought public attention to the disorder. Despite having knowledge of the symptoms and identification of these unusual cells within affected individuals, it was another thirty-nine years before the link to an aberrant hemoglobin protein structure was made by Dr. Linus Pauling (1). Advances in scientific technology have since allowed for the identification of the genetic mutation causing the alteration in the hemoglobin protein.

Role of Hemoglobin in Sickle Cell Anemia

Hemoglobin (Hb) is a globular protein found within red blood cells that coordinates the binding of oxygen molecules to heme groups allowing for the reversible binding of oxygen, carbon dioxide, carbon monoxide and nitric oxide gases for their transport throughout the body. The globular nature of the protein is dictated by the amino acid sequence, which dictates how the protein will be folded. While there are hundreds of types of hemoglobins, in normal red blood cells the majority of functional hemoglobin (97%) is a tetrameric complex comprised of two alpha and two beta globin proteins (1). Proper protein folding and formation of the tetrameric state is critical for the covalent association and protection of heme groups, iron-containing protoporphyrin molecules that coordinate the binding of the four aforementioned gaseous molecules. In normal hemoglobin, each of the four globin proteins has an associated heme group which enables it to carry four molecules of oxygen at once.

In Sickle Cell patients, this story is quite different. A single point mutation within the hemoglobin beta gene alters the amino acid sequence, and ultimately the secondary structure, of the protein so severely that the tetrameric globule is no longer maintained when hemoglobin is partially deoxygenated (**Figures 1 & 2**). Instead, linear structures that serve to distort the red blood cell are formed.

Gene: Normal: ACT CCT GAG GAG AAG
Sickle: ACT CCT GTG GAG AAG

Protein: Normal: MVHLTPEEKSAVT
Sickle: MVHLTPVEKSAVT

Figure 1. Mutation within the hemoglobin beta gene and the resulting alteration at the amino acid level.

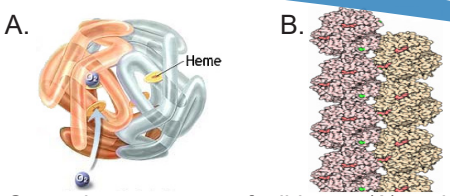


Figure 2. Secondary structure of wild type (A) and sickle cell (B) hemoglobin proteins. Oxygen-binding heme moieties are depicted. Obtained and modified from A.D.A.M. and Protein Data Base (PDB).

Genetics of Sickle Cell Anemia

Sickle Cell Anemia is an inheritable disease that is transmitted in an autosomal recessive manner, meaning two copies of the aberrant gene must be inherited in order to acquire the disease. Within the United States, there are an estimated 72,000 individuals afflicted with Sickle Cell Anemia which correlates with an incidence rate of 1 affected person in every 34,000 births. African Americans and Hispanics are affected at the highest rates, but individuals with familial ties to South and Central America, Cuba, India, Turkey, Greece and Italy are also genetically predisposed (2). The fact that this disorder is transmitted in an autosomal recessive manner and has such a high incidence rate suggests there is a rather high genetic carrier rate within these two ethnic populations. As a result, there are a number of possible hemoglobin β inheritance patterns that can occur, including Sickle Cell Disease, Sickle Cell Trait and Hemoglobin C Disease (Hb C), each with their own distinct clinical outcome.

Hb SS - People who have this form of sickle cell disease inherit two sickle cell genes (Hb S), one from each parent. This inheritance pattern is commonly referred to as “Sickle Cell Anemia” and is usually the most severe form of the disease.

Genetic Frequency:
African Americans: 1 in 500 (3)
Hispanics: 1 in 1,000 - 1,400 (3)

Hb SC - Sickle Cell Disease/Hemoglobin C Disease arising from the inheritance of one sickle cell gene (Hb S) and one Hb C gene; this combination usually results in a milder form of the disease characterized by minimal levels of anemia and mild to moderate enlargement of the spleen (4).

Genetic Frequency:
African Americans: 1 in 50-100 (3)

Hb AS – Sickle Cell Trait arising from the inheritance of one normal Hb and one Hb S gene. Individuals with sickle cell trait usually lead normal, asymptomatic lives but are capable of passing the disease on to their children.

Genetic Frequency:
African Americans: 1 in 12 (3)
Hispanics: 1 in 24 (3)

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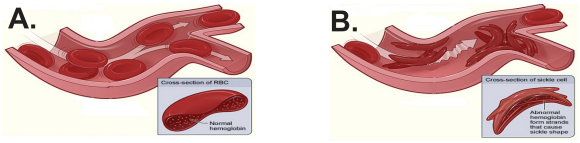
Symptoms of Sickle Cell Anemia

The main symptoms of the disease, anemia and pain, are the direct result of the production of this aberrant protein. Sickled red blood cells (RBC) have a much shorter lifespan, lasting only 10-20 days rather than the approximate 120 days observed for normal red blood cells. This, combined with the bone marrow's inability to regenerate the RBC population quickly enough, results in a severe anemia, characterized by shortness of breath, dizziness and headaches. The accompanying pain results from these abnormally shaped cells clogging small blood vessels, disrupting the normal flow of blood within limbs and organs (**Figure 2**). As a result, affected



individuals are susceptible to a host of secondary complications ranging from splenic crisis to stroke (5).

Figure 2. Flow of normal (A) and sickled (B) red blood cells through the vasculature. The altered shape of the sickled cells block small blood vessels, disrupting the normal flow of blood and inducing severe pain. Adapted from (6).

Diagnosis of Sickle Cell Anemia

Presently, more than 40 states have incorporated testing for Sickle Cell Anemia along with other routine newborn screening tests (2). By far, hemoglobin gel electrophoresis is the most common methodology used for diagnosis. This assay is based upon the association of novel electrophoretic banding patterns with a particular variant of the hemoglobin β proteins that would allow them to be differentiated from one another. In application, an electrical charge is applied to protein extracted from patient blood. As proteins migrate based on charge, a factor dictated by the amino acid sequence, variants become apparent (**Figure 3**). While there are many variants of hemoglobin, the example provided below is limited to those germane to the most predominant form of normal adult hemoglobin, Hb A, and those associated with sickle cell disease: Hb S and Hb C.

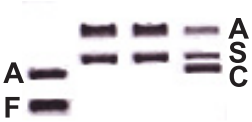


Figure 3. Hemoglobin typing by gel electrophoresis using the Beckman Coulter Paragon Acid Hb Gel assay. Abbreviations: A: Hb A, normal, adult hemoglobin; S: Sickle Hb; F: fetal Hb; C: Hb C.

While this approach is common place for the routine testing of newborns, the timing could prove to be problematic. At the time of birth, a specialized fetal form of hemoglobin predominates and the transition to adult hemoglobin, Hb A, does not fully occur until approximately the fourth month of life. Therefore, this early testing may not give the most complete picture as far as the patient's lifelong chances of inheriting a hemoglobinapathy or transmitting the disease to their offspring is concerned (7).

A drawback to the current methodologies of screening for Sickle Cell Anemia lies in the fact that they evaluate hemoglobin proteins rather than the actual gene. These current assays, marketed as Paragon, Titan III and Sickledex, are appropriate for the evaluation of a given individual but offer no information as far as the possibility of transmitting the disease to offspring. There are at least five variant forms of hemoglobin associated with various hemoglobinopathies, though many more variants have been identified. With this wide variance, electrophoresis-based assays are limited with respect to identifying each form. Based on the type of electrophoretic assay performed, alkaline or acidic, the banding patterns differ and interpretation is such that multiple variant hemoglobin proteins could be detected within a given region of the gel. The same banding pattern interpretation methodology is true for protein solubility and high pressure liquid chromatography (HPLC) based assay methods. In analyzing the hemoglobin gene for the specific point mutations linked to Sickle Cell Anemia, a more accurate risk of transmission to offspring assessment is now available.

Medical Diagnostic Laboratories (MDL) offers a genetic carrier screening assay, Test 1216: Sickle Cell Anemia by SNP Genotyping with Pyrosequencing, on its ***OneSwab***® platform that is designed to evaluate the hemoglobin β gene directly from cervicovaginal swabs. The assay identifiies the individual point mutations that manifest as the variant hemoglobins, S and C, by utilizing a combination of highly sensitive PCR in conjunction with Pyrosequencing to specifically amplify and sequence the DNA from both copies of the hemoglobin β gene (**Figure 4**). As such this assay is capable of not only identifying individuals who harbor the sickle cell mutation but can also identify individuals who are unaffected by the disease but carry an aberrant copy of the gene and, therefore, run the risk of transmitting a form of Sickle Cell Anemia to their offspring.

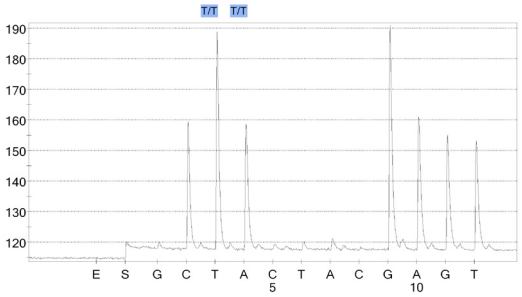


Figure 4. Pyrosequencing analysis of the hemoglobin beta (Hb B) gene. Result indicates normal hemoglobin B and mutant Hb C.

Treatments for Sickle Cell Anemia

There is no cure for Sickle Cell; the main form of treatment is the alleviation of pain and the prevention of complications linked to the increased susceptibility of infections, eye impairment and stroke. To achieve this, affected children are typically administered daily doses of antibiotics through the age of five as well as vaccinations against influenza, pneumococcus, hepatitis B and meningococcus in addition to the normal childhood regimens (3,4,5). Blood transfusions are administered to deal with bouts of anemia, as well as prevent spleen enlargement and recurrent strokes in children at highest risk (2).

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