

# Sickle Cell Anaemia and Genetics: Background Information

Background information to accompany the labs: *Allele Frequencies and Sickle Cell Anaemia Lab* and *Sickle Cell Anaemia: Diagnosis Using Restriction Analysis of DNA*

## Genetics of Sickle Cell Anaemia

Sickle cell anaemia was the first genetic disease to be characterized at the molecular level. The mutation responsible for sickle cell anaemia is small—just ONE nucleotide of DNA out of the three billion in each human cell. Yet it is enough to change the chemical properties of haemoglobin, the iron and protein complex that carries oxygen within red blood cells.

There are approximately 280 million haemoglobin molecules in each red blood cell (RBC). The protein portion of haemoglobin consists of four globin subunits: two alpha ( $\alpha$ ) and two beta ( $\beta$ ). These two types of subunits are encoded by the  $\alpha$  and  $\beta$  globin genes, respectively. While the binding of oxygen actually occurs at the iron sites, all four globin chains must work together in order for the process to function well.

Sickle cell anaemia, also known as sickle cell disease, is caused by a point mutation in the  $\beta$  globin gene. As a result of this mutation, valine (a non-polar amino acid) is inserted into the  $\beta$  globin chain instead of glutamic acid (an electrically charged amino acid). The mutation causes the RBCs to become stiff and sometimes sickle-shaped when they release their load of oxygen.

The sickle cell mutation produces a “sticky” patch on the surface of the  $\beta$  chains when they are not complexed with oxygen. Because other molecules of sickle cell haemoglobin also develop the sticky patch, they adhere to each other and polymerize into long fibers that distort the RBC into a sickle shape.

The sickled cells tend to get stuck in narrow blood vessels, blocking the flow of blood. As a result, those with the disease suffer painful “crises” in their joints and bones. They may also suffer strokes, blindness, or damage to the lungs, kidneys, or heart. They must often be hospitalized for blood transfusions and are at risk for a life-threatening complication called acute chest syndrome. Although many sufferers of sickle cell disease die before the age of 20, modern medical treatments can sometimes prolong these individuals’ lives into their 40s and 50s.

There are two  $\beta$  globin alleles important for the inheritance of sickle cell anaemia: A and S. Individuals with two normal A alleles (AA) have normal haemoglobin, and therefore normal RBCs. Those with two mutant S alleles (SS) develop sickle cell anaemia. Those who are heterozygous for the sickle cell allele (AS) produce both normal and abnormal haemoglobin. Heterozygous individuals are usually healthy, but they may suffer some symptoms of sickle cell anaemia under conditions of low blood oxygen, such as high elevation. Heterozygous (AS) individuals are said to be “carriers” of the sickle cell trait. Because both forms of haemoglobin are made in heterozygotes, the A and S alleles are codominant.

About 2.5 million African-Americans (1 in 12) are carriers (AS) of the sickle cell trait. People who are carriers may not even be aware that they are carrying the S allele!

## **Sickle Cell Anaemia and Malaria**

In the United States, about 1 in 500 African-Americans develops sickle cell anaemia. In Africa, about 1 in 100 individuals develops the disease. Why is the frequency of a potentially fatal disease so much higher in Africa?

The answer is related to another potentially fatal disease, malaria. Malaria is characterized by chills and fever, vomiting, and severe headaches. Anaemia and death may result. Malaria is caused by a protozoan parasite (*Plasmodium*) that is transmitted to humans by the *Anopheles* mosquito.

When malarial parasites invade the bloodstream, the red cells that contain defective haemoglobin become sickled and die, trapping the parasites inside them and reducing infection. Compared to AS heterozygotes, people with the AA genotype (normal haemoglobin) have a greater risk of dying from malaria. Death of AA homozygotes results in removal of A alleles from the gene pool. Individuals with the AS genotype do not develop sickle cell anaemia and have less chance of contracting malaria. They are able to survive and reproduce in malaria-infected regions. Therefore, BOTH the A and S alleles of these people remain in the population. SS homozygotes have sickle cell anaemia, which usually results in early death. In this way, S alleles are removed from the gene pool.

In a region where malaria is prevalent, the S allele confers a survival advantage on people who have one copy of the allele, and the otherwise harmful S allele is therefore maintained in the population at a relatively high frequency. This phenomenon will be examined in the

### **Allele**

**Frequencies and Sickle Cell Anaemia Lab**, which relates the change in allele frequency in a population to evolution. The frequency of the S allele in malaria-infected regions of Africa is 16%. The sickle cell allele is also widespread in the Mediterranean and other areas where malaria is or used to be a major threat to life. In contrast, the S allele frequency is only 4% in the United States, where malaria has been virtually eliminated. Malaria was once common in the United States, but effective mosquito control caused the number of cases to drop. Recently, however, there has been an increase in the number of malarial cases because of increased travel, immigration, and resistance to medication. In Southern California there was a 1986 outbreak of nearly 30 cases of malaria transmitted by local mosquitoes!

### **Sickle Cell Anaemia and Current Research**

The oxygen requirements of a fetus differ from those of an adult, and so perhaps not surprisingly, prenatal blood contains a special haemoglobin. Foetal haemoglobin contains two gamma (©) globin polypeptide chains instead of two adult ® chains. After birth, the genes encoding © globin switch off, and the ones encoding ® globin switch on. Understanding how this genetic switch works could allow researchers to understand much about the control of genes in general and sickle cell anaemia in particular.

Indian and Saudi Arabian people have a milder variation of sickle cell anaemia, sometimes with no symptoms. In this population twenty-five percent of each person's haemoglobin is the foetal kind.

Similarly, the blood of adults with an inherited condition called “hereditary persistence of foetal haemoglobin” also contains foetal haemoglobin and these individuals are healthy. Some people with this condition completely lack adult haemoglobin and still show no ill effects. Biochemical experiments have demonstrated that, in a test tube, foetal haemoglobin inhibits polymerization of sickle cell haemoglobin. These observations suggest that increasing foetal haemoglobin levels may be an effective treatment for sickle cell anaemia. There are a number of lines of research related to activation of foetal haemoglobin as a therapy for sickle cell anaemia:

- Some infants whose mothers suffered from diabetes during pregnancy have unusually high concentrations of the biochemical butyrate in their blood plasma. Butyrate is a natural fatty acid that stimulates RBCs to differentiate from their precursors (reticulocytes). Butyrate also prevents the  $\gamma$  globin gene from switching off and the  $\beta$  globin gene from switching on in these infants, who are healthy despite lacking adult haemoglobin. When butyrate is given to patients with sickle cell anaemia, the  $\gamma$  globin mRNA levels in reticulocytes increase significantly.

Perhaps butyrate or other chemicals that stimulate foetal haemoglobin production could be used to treat sickle cell anaemia.

- In 1983, a drug called hydroxyurea (HU) was first used on sickle cell patients to try to activate their foetal globin genes. By 1995, clinical trials had demonstrated that HU could increase foetal haemoglobin levels in patients’ RBCs and prevent the cells from sickling. Patients treated with HU experienced less frequent and severe painful crises. However, hydroxyurea can be quite toxic when used continuously to maintain elevated levels of foetal haemoglobin and can increase the risk of leukaemia.

- In 1992, it was found that alternating hydroxyurea with erythropoietin and providing dietary iron raised the percentage of RBCs with foetal haemoglobin and relieved the joint and bone pain of sickle cell disease. Erythropoietin is made in the kidneys and helps anaemic patients replenish their RBCs. It can be manufactured for therapeutic use with recombinant DNA technology.

- Mice that have been genetically engineered to contain a defective human  $\beta$  globin gene have symptoms typical of sickle cell anaemia, making them an ideal model for laboratory experimentation. In 2000, these mice were mated to another transgenic mouse line expressing human foetal haemoglobin. When compared to their sickle cell parents, the offspring had greatly reduced numbers of abnormal and sickled RBCs, increased numbers of RBCs overall (reduced anaemia), and longer life spans. These experiments established that only 9-16% of haemoglobin need be the foetal type in order to ameliorate the sickle cell symptoms, and are an important first step in a gene therapy solution to sickle cell disease.

### **Disclaimer:**

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