Sickle Cell Anemia: Example of a Point Mutation

Now that the actual nucleotide sequences of normal and mutant alleles of many genes from humans and other organisms have been determined, we could use numerous examples to show how a mutant allele can lead to the resulting phenotype. However, for illustrative purposes, we will examine only one case in detail. The example we will use, sickle cell anemia, was the first case where all the molecular information became available. Unfortunately, in this case as opposed to examples like phenylketonuria, understanding the molecular changes that take place did not suggest a useful treatment.

Sickle cell anemia gets its name from the shape of red blood cells in affected individuals. They tend to collapse and give the cells a crescent or sickle appearance in contrast to the doughnut-like appearance of normal cells. A photo of blood cells from a patient can be seen at: http://www-medlib.med.utah.edu/WebPath/HEMEHTML/HEME062.html.

You may be aware that sickle cell is more common among blacks than whites; the reasons for this should become clear as we proceed.

Sickle cell anemia is inherited as a simple recessive condition. As is the case with thalassemia, the anemia is so severe that it is normally lethal by the teens, although life can be extended by blood transfusions and antibiotics. Likewise, heterozygous individuals are carriers, but do not have anemia.

Recognition that abnormal blood cells were involved gave scientists some clues as to the genes that may be involved. When hemoglobin from normal and affected individuals was extracted from red cells and subjected to electrophoresis, a procedure for separating charged molecules, the hemoglobins migrated to different positions. Patients are said to have Hb-S rather than the usual Hb-A found in adults. In fact, as we will see, the defective gene in sickle cell affects the gene that codes for the beta chain of adult (alpha2, beta2) hemoglobin. Rather than A and a, the normal Hb-beta allele is referred to as "A", while the allele in sickle cell patients is designated "S". Several characteristics of normal, heterozygous and homozygous affected individuals are given in the table on the next page. As you can see, it is possible to detect carriers, so that the odds of a couple having an affected child can be determined even
before the first child is born. Later in the course, we will describe procedures that permit prenatal detection to be made, even though the defective gene is not expressed in a fetus.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Red Cell Shape</th>
<th>Red Cell Shape in a Vacuum</th>
<th>Malaria</th>
<th>Electrophoresis</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Hb^A_{\beta} Hb^A_{\beta}$</td>
<td>○</td>
<td>○</td>
<td>Susceptible</td>
<td>i</td>
<td>AA</td>
</tr>
<tr>
<td>$Hb^S_{\beta} Hb^A_{\beta}$</td>
<td>○</td>
<td>○ &amp; &amp;</td>
<td>Resistant</td>
<td>i</td>
<td>A/S</td>
</tr>
<tr>
<td>$Hb^S_{\beta} Hb^S_{\beta}$</td>
<td>🍁</td>
<td>🍁</td>
<td>----</td>
<td>i</td>
<td>SS</td>
</tr>
</tbody>
</table>

The observation that heterozygotes are resistant to malaria means that they actually have a reproductive advantage in areas of the world where malaria is a problem. This is true even though 1/4 of the children of two heterozygous parents will inherit a lethal disease.

The perfect correlation between the presence of sickle cells with a variant type of globin led Hunt and Ingram in the 1950's to investigate the basis for the differences in the two types of globin. They found that when the hemoglobin proteins were first separated and then subjected to electrophoresis, only the $\beta$-chains of Hb-A and Hb-S were different. Each of the $\beta$-chains is 146 amino acids in length, so the next problem was to compare the two sequences. By this time, purified proteases (enzymes that digest proteins by breaking peptide bonds) had been found, and shown to recognize specific cutting sites. For example, the enzyme trypsin cuts the chain of amino acids after each arginine and lysine whereas chymotrypsin cleaves before tyrosine and phenylalanine. When beta-globin samples from Hb-A and Hb-S blood were digested, the fragments produced were spotted onto filter paper and "fingerprinted" by separation in 2 dimensions. Separation in one direction used electric current and the other used chromatography with well-defined solvents. When the fragments were stained for viewing, both the HbA and HbS
samples had 30 spots, 29 of which were identical. The unique fragments would seem to hold the key.

Just as enzymes (proteases) that cleave peptide bonds within a chain of amino acids had been purified, others that nibbled away from the amino- and carboxy-terminal ends of a chain of amino acids were also available. By slowly and carefully digesting away and identifying the amino acid released from each end, the entire sequence of short peptide fragments could be determined. Hunt and Ingram found that the unique beta globin fragment from Hb-A had the sequence Val-his-leu-thr-pro-glu-glu-
while that from sickle cell anemia was Val-his-leu-thr-pro-val-glu-

Since glu has a negative charge and val is neutral, this single amino acid difference (glu -> val) accounts for the difference in mobility during electrophoresis. Eventually, all the fragments were sequenced and could be put in proper order by overlapping the ends of fragments produced when a different protease was used in the initial digestion. The fragment sequences shown above turned out to be the first seven amino acids (N-terminal) of the beta-globin chain. Thus it was shown that the difference between sickle cell and normal individuals can be traced to a missense mutation that causes a single amino acid substitution at position 6 in the beta-globin protein.

What are the consequences of this change? Red cells that contain the abnormal globin tend to collapse, so they do not carry oxygen well. This causes the patient to attempt to make more hemoglobin. Extra bone marrow is made, leading to fragile bones and tower skull. The heart also enlarges to try to improve oxygen transport by pumping more blood. The anemia itself causes weakness and poor mental and physical development. The abnormal sickle cells are collected in the spleen which enlarges and becomes fibrotic. Most serious are problems caused by clumping of sickle cells, which tends to clog capillaries and cause local failures of blood supply. Patients often have episodes of severe abdominal or joint pain as a consequence. The lack of adequate blood supply causes kidney damage, strokes, heart damage and frequent cases of pneumonia. The only remedy for years was transfusions, but this provided only temporary relief and leads to accumulation of toxic levels of iron. Two chemicals (hydroxyurea and butyrate) are being tested that seem to be able to help by turning on expression of fetal hemoglobin to some extent in many patients. Long term consequences of using these
compounds have yet to be determined. More promising may be the use of bone marrow transplant or introduction of cells from fetal tissues that do not induce antibody responses, but these are also experimental treatments at this time.

This one example shows how remarkable the consequences of a single base change in the DNA can be; even though the mutant allele is lethal when homozygous, the fact that heterozygotes are resistant to malaria has kept the allele in the human population.