Sickle Cell Hemoglobin and Malaria

Natural Selection in Human Populations

Natural Selection

- Darwinian natural selection is a two-step process:
  - The production of new genetic variation through the process of mutation
  - The differential reproduction of favorable variants through the process of selection

Selection

- Selection causes changes in allele and genotype frequencies from one generation to the next due to differential net reproductive success of individuals with different genotypes
  - If individuals with genotype AA consistently have more offspring on average than individuals with AB and BB genotypes, the frequency of the A allele will increase through time and eventually, everyone will have the AA genotype, and the B allele will be lost from the population

Structure of Hemoglobin

- 4 polypeptides (globins) and 4 iron-based oxygen-binding heme molecules

Genetics of Hemoglobin

- The alpha and beta globin genes are transmitted separately, as parts of the 16th and 11th chromosome pair, respectively
  - Transmission follows Mendel’s Law of Independent Assortment for genes located on different homologous chromosome pairs
  - Each individual has two alpha globin genes and two beta globin genes inheriting one gene of each pair from the mother and one from father

Function of Hemoglobin

- Hemoglobin is the primary protein constituent of red blood cells
  - Transports oxygen by binding with it tightly as the red blood cells pass through the capillaries of the lungs
  - As oxygenated red blood cells circulate through the heart and to the other body tissues, hemoglobin loosens its hold on the oxygen so that it can pass readily out of the red cells and be made available to peripheral cells for respiration
**Function of Hemoglobin 2**

- Deoxygenated hemoglobin molecules in peripheral capillaries bind loosely with carbon dioxide and help remove this waste product of cellular respiration to the lungs for expiration.
- The binding capacity of hemoglobin molecules is a function of the partial pressure of oxygen in the blood.
  - high in the lungs, bind tightly
  - low in peripheral capillaries, bind loosely

**Hb^A**

- “Wild” or most common form, found in all human populations.
- Beta Hemoglobin: 6\text{th} \hspace{1em} 26\text{th}
- DNA sequence: C-T-C \hspace{0.25em} C-T-C
- Amino Acids: Glutamic Acid \hspace{0.25em} Glutamic Acid

**Hb^S**

- Sub-Saharan Africa, Mediterranean, Middle East, South Asia.
- Beta Hemoglobin: 6\text{th} \hspace{1em} 26\text{th}
- DNA sequence: C-A-C \hspace{0.25em} C-T-C
- Amino Acids: Valine \hspace{0.25em} Glutamic Acid

**Hb^C**

- Predominantly West African populations.
- Beta Hemoglobin: 6\text{th} \hspace{1em} 26\text{th}
- DNA sequence: T-T-C \hspace{0.25em} C-T-C
- Amino Acids: Lysine \hspace{0.25em} Glutamic Acid

**Sickled vs. Normal RBC**

- Sickled cell versus normal red blood cell.

**Sickle Cell Disease in the United States**

- 289/100,000 is equivalent to approximately 0.3% of the African-American population having Sickle Cell Anemia, or an S allele frequency of about 5% for African-Americans.
- Predominantly White Hispanic, Asian American, Native American.
**Hb\textsuperscript{E}**

- Southeast Asia
- Beta Hemoglobin
  - 6\textsuperscript{th} 26\textsuperscript{th}
  - DNA sequence C-T-C T-T-C
  - Amino Acids Glutamic Acid Lysine

**Genetics of Sickle Cell**

- Three genotypes can form from combinations of the A and S alleles
  - AA Homozygous Normal; “Normal”
  - AS Heterozygote; “Sickle Cell Trait”
  - SS Homozygous sickler; “Sickle Cell Anemia”
  - Inheritance follows a Mendelian pattern for a co-dominant autosomal allele
    - When two heterozygotes mate, ¼ of their offspring are predicted to be SS and have sickle cell anemia, ¼ AA normal, and ½ AS, carrying the sickler allele

**Sickling**

- Red blood cells begin to sicken when hemoglobin molecules have given up their oxygen in the capillaries
- The S-hemoglobin molecules bind together into long fibers, forming a complex helical molecule within the red blood cell

**Sickling of Cells**

**Symptoms of Sickle Cell Anemia**

- As a result of sickling and the premature aging of red blood cells from sickling, there are fewer than normal red blood cells, the general condition referred to as anemia
- There is an increased risk of severe infections, especially bacterial infections--such as sepsis (a blood stream infection), meningitis, and pneumonia, especially in early childhood
  - The risk of infection is increased because the spleen does not function normally

**Symptoms 2**

- Splenic sequestration crisis:
  - The spleen is the organ that filters blood
  - In children with sickle cell disease, the spleen can enlarge rapidly from trapped red blood cells creating a situation that can be life-threatening.
- Stroke:
  - This happens when blood vessels in the brain are blocked by sickled red blood cells
  - Signs include seizure, weakness of the arms and legs, speech problems, and loss of consciousness.
Symptoms 3

- Children with sickle cell anemia experience slowed growth and delayed maturation, including puberty as a result of the anemia and infections.
- There are repeated, painful episodes, called vaso-occlusive crises, associated with blockages of the circulatory system.
  - Frequently seen as swelling of extremities.
- There is a progressive degeneration of organs from impaired circulation.

Blockage of Capillaries

Swollen Hands from Sickling

Malaria

- Host: Man, although higher primates can also may harbor *P. malariae*.
  - Monkeys harbor other plasmodium species which can infect man.
- Symptoms: Cyclic (sometimes) high fever, chills and sweating, headache, coagulation defects, shock, anemia (inducing jaundice), kidney failure, acute encephalitis, coma.

Malaria Cycle of Infection
Sickle Cell and Malaria

- There are three lines of evidence suggesting an association between the sickle cell allele and Malaria:
  - Geographic correlations
  - Epidemiological associations
  - Biochemical studies

Geographic Correlations

- Clinal studies show a substantial overlap between the distribution of malaria in the and the frequency of the sickle cell allele
  - The Malaria belt extends across the Southern Mediterranean, sub-Saharan Africa, the Middle East, India, Southeast and Island Southeast Asia, and Northern Australia, the S allele exceeds a frequency of 10% only in Africa
  - In the Mediterranean β thalassemia and G-6-PD are elevated, while in India and Southeast Asia, Hemoglobin E and α thalassemia predominate

Epidemiological Correlations

- A comparison of *Plasmodium falciparum* parasites in blood samples from children of AA and AS genotypes in Nigeria to that of children with AA genotypes showed:
  - AS children had lower frequencies and lower densities of parasites than AA children
  - Fertility did not differ between AA and AS, but 29% more AS individuals survived to adulthood

Biochemical studies

- *Plasmodium* metabolism causes sickle cell hemoglobin to form the fibers that results in red blood cell sickling
  - The parasite significantly increases RBC acidity causing hemoglobin to release oxygen
  - “Knobs” form on the cell wall of infected RBCs slowing transit through capillaries
  - The result is an increase of sickling to about 40% of the RBCs in AS heterozygous individuals
  - Sickling of the RBC causes a disruption in the reproductive cycle of *Plasmodium*, resulting in lower parasite levels and less severe symptoms
Diet and Sickle Cell

- Consumption of yams, common in many sickle cell areas of Africa, results in a build up of cyanide compounds that reduce the severity of sickling
- These same compounds are thought to be a partial prophylactic against malaria for individuals with normal hemoglobin
  - This is due to the action of the compounds on the G6PD dependent pathways

Glucose-6-Phosphate Dehydrogenase (G6PD) and Malaria

Structure of G6PD

- The enzyme, Glucose-6-Phosphate Dehydrogenase, is comprised of a dimer or tetramer of identical polypeptide chains
  - Each unit consists of 515 amino acids
- The single G6PD locus in humans is located on the telomeric region of the long arm of the X-chromosome
  - Females have two X chromosomes, hence two copies of G6PD, while males have only one X chromosome and one copy of G6PD

Function of G6PD

- G6PD is present in the cytoplasm of all cells of the body
  - In Red Blood Cells (RBC), which lack nuclei, mitochondria, and other organelles, G6PD is particularly significant
    - G6PD is involved in the first step of the Pentose Phosphate Shunt
      - Catalyzes the oxidation of Glucose-6-Phosphate to 6-Phosphogluconolactone (Phosphogluconate)
      - Only source of NADPH and GSH, necessary for the reduction of hydrogen peroxide
    - Hydrogen Peroxide is a strong oxidant that will degrade the RBC and cause hemolysis if it is not reduced
Familial Genetics of G6PD

- Five genotypes can form from combinations of one normal (GdB) and one deficient form (e.g., GdA- or GdMed) of G6PD
  - **Females**
    - GdB GdB, Homozygous Normal; “Normal”
    - GdB GdA-, Heterozygous; “Heterozygote”
    - GdA- GdA-, Homozygous Deficient; “G6PD Deficient”
  - **Males**
    - GdB, Hemizygous Normal; “Normal”
    - GdA-, Hemizygous Deficient; “G6PD Deficient”

Symptoms of G6PD deficiency

- G6PD deficiency is manifested as anemia, with RBCs being prematurely destroyed
  - RBCs are also extremely susceptible to oxidative stress
  - Neonatal jaundice is a yellowish discoloration of the whites of the eyes, skin, and mucous membranes caused by deposition of bile salts in these tissues
    - A severe form of this is a direct result of insufficient activity of the G6PD enzyme in the liver
      - In some cases, the neonatal jaundice is severe enough to cause death or permanent neurologic damage (Beutler, 1994).

G6PD Hemolysis

- Red blood cells will hemolyze or burst when the oxidant stress level becomes too high
  - Hemolysis occurs in G6PD deficient individuals due to the consumption of certain foods or drugs
    - Substances that increase the oxidation of glutathione, thereby diminishing the available GSH for oxidation of peroxide, creating a potential for hemolysis
      - Fava Beans contains vicine and convicine whose metabolites can cause a hemolytic crisis in GdMed individuals
      - Many anti-malarial drugs, sulfonamides, sulfones and other drugs produce the same reaction in severely deficient individuals
      - Can also cause the oxidation of hemoglobin, making it lose the ability to be a reversible oxygen carrier

Favism

- The Fava Bean (Vicia faba) is a favored cultigen in areas where the GdMed allele is common
  - Vicine and convicine make up about 0.5% of the wet weight of the Fava bean
    - These compounds metabolize to divicine and isouramil in the intestine
      - These metabolites decrease RBC reduced glutathione (GSH)
      - Increase the production of hydrogen peroxide and free radicals
      - Creates a severe oxidant stress in G6PD deficient cells

**G6PD and Fava Beans**

- **G6PD and Fava Beans**

Plasmodium in the RBC

- *Plasmodium* protozoans preferentially attack immature RBC but *P. falciparum* can invade RBC of all ages
  - *Plasmodium* oxidizes RBC NADPH from the Pentose Phosphate pathway for its metabolism
    - This results in a deficiency of RBC GSH, most severe in G6PD deficient individuals, leading to peroxide-induced hemolysis which curtails the development of *Plasmodium*
      - After several cell cycles the *Plasmodium* can adapt to produce its own G6PD, reducing the adaptive benefit of G6PD deficiency
Glucose-6-Phosphate Dehydrogenase (G6PD) and Malaria

Recall that fava beans contain compounds that metabolize to powerful oxidants.

- In a cell that is oxidant-stressed by Plasmodium infection, the addition of another strong oxidant can lead to a rapid build-up of peroxide.

- *In vitro* and *in vivo* (mouse) studies indicate a mild suppressant effect of divicine and isouramil on *Plasmodium* in G6PD normals.

  - This effect is even greater in G6PD deficient individuals.