Infectious Disease

- Major cause of morbidity and mortality until 20th century in all cultures
- Important cause of morbidity and mortality in many world populations in 21st century

Major Classes of Infective Agents

- Bacteria
- Viruses
- Protozoa (Single cell eukaryotic organisms)
- Fungi
- Parasitic worms
- Prions
Mortality in Early Neolithic Agricultural Society

Impact of Agriculture on Infectious Disease

- Rise in human population density
- Permanent population centers
- Domestic animals create new source for infectious agents
- Permanent food storage sites provide habitat for commensal species such as rats and mice which were an additional source of new infectious agents
Some Major Human Infectious Diseases and their Most Likely Animal Source

- Measles--cattle (rinderpest)
- Tuberculosis--cattle
- Smallpox--cattle or other livestock with related poxviruses
- Influenza--pigs and ducks
- Falciparum malaria--chickens or ducks

Mortality in London 1700s
Causes of Death in 17th Century London

- Infant mortality approximately 50%
- Likelihood of death between age 20 and age 50 approximately 50%
- Infectious disease account for approximately 66% of deaths
- Cancer and heart disease are not important contributors to death rates

Infant Mortality

- Diarrheal diseases are a major cause of infant mortality
- Transmitted as a consequence of poor sanitation
- Death occurs because of dehydration and electrolyte loss
- Lethal effects can be mitigated by restoring water and electrolyte balance during infection
Mortality in United States 1980s

Changes in Death Rates in 20th Century in United States

- Infectious disease a minor cause of death
- Improved sanitation and high rates of vaccination major reasons infectious disease rates have dropped
- Cancer, heart disease and stroke responsible for more than 70% of all deaths
## Causes of Death in the US in 2003

<table>
<thead>
<tr>
<th>Rank</th>
<th>Causes of death</th>
<th>All persons</th>
<th>Causes of death</th>
<th>Male</th>
<th>Causes of death</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diseases of heart</td>
<td>686,947</td>
<td>Diseases of heart</td>
<td>340,005</td>
<td>Diseases of heart</td>
<td>356,942</td>
</tr>
<tr>
<td>2.</td>
<td>Malignant neoplasms (cancer)</td>
<td>557,271</td>
<td>Malignant neoplasms (cancer)</td>
<td>289,788</td>
<td>Malignant neoplasms (cancer)</td>
<td>268,503</td>
</tr>
<tr>
<td>3.</td>
<td>Cardiovascular diseases</td>
<td>162,672</td>
<td>Unintentional injuries</td>
<td>69,257</td>
<td>Cardiovascular diseases</td>
<td>100,050</td>
</tr>
<tr>
<td>4.</td>
<td>Chronic lower respiratory diseases</td>
<td>124,916</td>
<td>Cardiovascular diseases</td>
<td>62,022</td>
<td>Chronic lower respiratory diseases</td>
<td>64,103</td>
</tr>
<tr>
<td>5.</td>
<td>Unintentional injuries</td>
<td>106,742</td>
<td>Chronic lower respiratory diseases</td>
<td>66,713</td>
<td>Alzheimer's disease</td>
<td>41,877</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetes mellitus</td>
<td>75,946</td>
<td>Diabetes mellitus</td>
<td>54,301</td>
<td>Diabetes mellitus</td>
<td>38,948</td>
</tr>
<tr>
<td>7.</td>
<td>Influenza and pneumonia</td>
<td>68,681</td>
<td>Influenza and pneumonia</td>
<td>28,918</td>
<td>Unintentional injuries</td>
<td>37,485</td>
</tr>
<tr>
<td>8.</td>
<td>Alzheimer's disease</td>
<td>56,866</td>
<td>Suicide</td>
<td>26,406</td>
<td>Influenza and pneumonia</td>
<td>38,783</td>
</tr>
<tr>
<td>9.</td>
<td>Nephritis, nephrotic syndrome, and nephrosis</td>
<td>40,974</td>
<td>Nephritis, nephrotic syndrome, and nephrosis</td>
<td>15,655</td>
<td>Nephritis, nephrotic syndrome, and nephrosis</td>
<td>21,279</td>
</tr>
<tr>
<td>10.</td>
<td>Septicemia</td>
<td>33,866</td>
<td>Chronic liver disease and cirrhosis</td>
<td>17,451</td>
<td>Septicemia</td>
<td>16,415</td>
</tr>
</tbody>
</table>


## Causes of Death Worldwide Comparing Developing Countries to Developed Countries

### Leading Causes of Death in 2001

<table>
<thead>
<tr>
<th>Developing Countries</th>
<th>Number of Deaths</th>
<th>Developed Countries</th>
<th>Number of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HIV/AIDS</td>
<td>2,678,000</td>
<td>1. Ischaemic heart disease</td>
<td>3,512,000</td>
</tr>
<tr>
<td>2. Lower respiratory infections</td>
<td>2,643,000</td>
<td>2. Cerebrovascular disease</td>
<td>3,348,000</td>
</tr>
<tr>
<td>3. Ischaemic heart disease</td>
<td>2,484,000</td>
<td>3. Chronic obstructive pulmonary disease</td>
<td>1,829,000</td>
</tr>
<tr>
<td>4. Diarrhoeal diseases</td>
<td>1,793,000</td>
<td>4. Lower respiratory infections</td>
<td>1,183,000</td>
</tr>
<tr>
<td>5. Cerebrovascular disease</td>
<td>1,381,000</td>
<td>5. Trachea/bronchus/lung cancers</td>
<td>938,000</td>
</tr>
<tr>
<td>6. Childhood diseases</td>
<td>1,217,000</td>
<td>6. Road traffic accidents</td>
<td>669,000</td>
</tr>
<tr>
<td>7. Malaria</td>
<td>1,033,000</td>
<td>7. Stomach cancer</td>
<td>657,000</td>
</tr>
<tr>
<td>8. Tuberculosis</td>
<td>1,021,000</td>
<td>8. Hypertensive heart disease</td>
<td>635,000</td>
</tr>
<tr>
<td>9. Chronic obstructive pulmonary disease</td>
<td>746,000</td>
<td>9. Tuberculosis</td>
<td>571,000</td>
</tr>
<tr>
<td>10. Measles</td>
<td>674,000</td>
<td>10. Sui-inflicted</td>
<td>498,000</td>
</tr>
</tbody>
</table>

Source: WHO World Health Report 2002. Countries grouped by WHO Mortality Stratum, with Developing Countries representing regions with High and Very High Mortality, and Developed Countries representing regions with Low and Very Low Mortality.
Leading infectious killers

Millions of deaths, worldwide, all ages, 1998

Genetic Mutation and Selection are Key in the Interaction between Host and Pathogen

- Selection of better adapted variants of the pathogen
- Selection of genetically resistant hosts
- Selection of genetic variation in cells of the adaptive immune system to produce effective antibodies and cellular immunity against pathogens
An example of genetic selection of both host and pathogen

In 1950 Australia attempted to control the rabbit population by introducing the highly virulent myxoma virus: at first the rabbit population plummeted, but within a few years the rabbits had become genetically resistant to the virus and the virus became attenuated.

Infectious Disease: Some Examples from History

- Plague of Athens (430 BC)
- Described by Thucydides in History Of Peloponnesian Wars
- Plague brought to Athens by ship from Egypt
- Symptoms described do not match any known infectious disease
- Siege of Athens provided ideal breeding grounds for infectious disease
- Kills 33% of population in two years
Infectious Disease: Some Examples from History

- 79 AD  Malaria devastates Rome, thousands die, fertile farmland abandoned, malaria becomes endemic to Italy
- 125 AD  Plague of Orosius kills millions; symptoms suggest measles as a possible cause
- 166 AD  Smallpox kills 4-7 million people in Europe over a 6 year period
- 250 AD  Plague of Antoninus kills up to 5000 Romans a day, causative agent uncertain

Smallpox and measles: childhood diseases which kill adults

- Smallpox and measles are diseases which are generally survived by children but are extremely lethal to adults
- A communicating population of at least 500,000 is necessary to maintain these diseases as childhood illnesses
- During 1-500 AD, these diseases “ping-ponged” between major world population centers in Europe and Asia until they became firmly established as childhood diseases
Justinian’s plague --542 AD

• Bubonic plague comes to Constantinople--up to 10,000 people per day die--40% of the population die
• Plague spreads to Italy, Spain, France, Britain and northern Europe, China, India, southeast Asia--death rates are high
• Rats and fleas carried by the rats transmit the plague

Return of Bubonic Plague: the Black Death

• Bubonic plague circulates throughout Europe and Asia from 542 AD onward for over a hundred years but then recedes
• Plague reappears in India, China and central Asia in 1344-1346
• Enters Europe in 1346 during siege of Tartars
• Kills 25-50% of population of Europe; death rates in Islamic world and China equally high
• Bubonic plague returns to human populations from endemic foci of indigenous rodents
Malaria--Worldwide Impact

- **40% of the world's population** - mostly those living in the poorest countries - is at risk of malaria.
- Malaria causes between **300 - 500 million cases of acute illness** and over **1 million deaths** annually.
- **90% of deaths due to malaria occur in Africa**, south of the Sahara desert - mostly amongst young children.
- Malaria is the number one killer of young children in Africa, accounting for **1 in 5 of all childhood deaths**

Infection begins when the mosquito injects *sporozoites* directly into the blood stream, within a few minutes they reach the liver, where they invade cells and become a hepatic *trophozoite* (feeding stage), this grows quickly and divides internally to give an hepatic *schizont* which contains many thousands of tiny, invasive *merozoites*. 
The merozoites are the smallest and shortest lived form of the life cycle, and within a few minutes they invade a red blood cell. The apical complex of the merozite is specialized to recognise and attach to specific molecules on the surface of the red blood cell.

The parasite produces proteins that become incorporated into the parasitophorous membrane and into the surface membrane of the rbc, so infected rbc's express on their surface parasite derived proteins. The growing trophozoite is amoeboid and ingests haemoglobin which is broken down to give an iron heme pigment called *haemozoin* which accumulates in the food vacuoles.
After about 30-40 hours growth in the red blood cell, schizogony begins (2nd asexual stage), and the trophozoites divide to give 8-16 merozoites which are released when the rbc ruptures. It is the synchronous rupture of red blood cells which gives the periodic fever, and the haemozoin (malarial pigment) which is released when the rbc's rupture is believed to be responsible for the fever.

After several blood cycles, a proportion of the trophozoites develop not into merozites, but into gametocytes, these take about 4 days to mature, but can then stay viable in the blood for prolonged periods. Nothing happens to the gametocytes unless they are taken up by a mosquito in a blood meal. However, within a few minutes of ingestion by a mosquito dramatic changes take place in the gametocytes.
Both gametocytes swell and burst out of the erythrocyte. The male gametocyte produces 8 *microgametes*, which consist of a flagellum with an attached nuclear mass. The micro and macrogametes fuse forming a zygote. This is the only stage in the *Plasmodium* life cycle that is diploid, all the other stages are haploid.

Over a period of 5 to 10 hours the zygote differentiates into a cigar-shaped motile invasive *ookinete*. This can penetrate either between or through the intestinal cells of the mosquito and comes to rest between the mid-gut cells and the basement membrane. During the differentiation of the ookinete the diploid genome divides as the first step in a two stage meiosis, the second stage takes place at the start of sporogony.
The embedded ookinete becomes an oocyst which grows rapidly and divides internally into sporozoites - the third asexual phase. The oocyst is the longest phase in the life cycle and is very dependent on the temperature, 8-35 days. The mosquito has to survive long enough for the oocyst to mature before it can infect anyone. So only elderly mosquitoes can pass on malaria and in the wild of course many mosquitoes never reach old age. This is where temperature matters, the warmer the weather, the faster the oocyst can develop in the mosquito. Mosquito survival is probably the single most important factor in malaria transmission.

Each oocyst produces up to 1000 sporozoites, when the oocyst bursts the sporozoites are released into the body cavity of the mosquito. They migrate anteriorly to the salivary glands, where they penetrate the basement membrane, pass through the cells and accumulate in the salivary ducts. The cycle is completed when the mosquito bites a susceptible host.
Plasmodium falciparum has a very broad worldwide distribution. It is responsible for most malaria fatalities.

Infected red blood cells develop surface 'knobs' which cause them to stick to endothelial cells (cells lining blood vessels). This causes blockages and brain and intestinal damage, often resulting in death, which can occur within a few days of infection.

*P. falciparum* is especially dangerous to small children and to travellers from non-malarious areas. There is no dormant hypnozoite (liver) stage. This feature is characteristic of avian malarias. Because immunity is poor, an individual may be reinfected multiple times.

DNA sequence analysis suggests that *P. falciparum* is related to avian malarias and has recently become adapted to humans.
Plasmodium Vivax is a common form of malaria today. It has a tertian (48 hour) fever period. The dormant stages called hypnozoites can persist in the liver for several years and cause relapses. It does not have the degree of lethality associated with P. falciparum.

P. malariae is less common, today than P. vivax or P. falciparum. It has a quartan (72 hour) pattern of fevers. The dormant hypnozoite stage can persist for up to thirty years. Lethality is much lower than P. falciparum.
Plasmodium ovale is found primarily in West Africa. It has a tertain (48 hour) fever period. The dormant stages called hypnozoites can persist in the liver for several years and cause relapses. It does not have the degree of lethality associated with p. falciparum.

What about immunity?

- Adults from areas where malaria is endemic develop a form of partial immunity.
- This partial immunity develops slowly and only in response to repeated infections.
- In partially immune people, malaria parasites can often be found in the blood, but without clinical symptoms.
- Immunity is lost if exposure is not maintained. (after 6 months).
What about immunity?

- There have been serious efforts to develop a low cost and effective malaria vaccine
- No vaccine has proven effective to date

By 1970 malaria eradication had succeeded with DDT in all Europe. Malaria used to be prevalent in Russia, USA & Canada. It was prevalent around the Mediterranean, Italy, Greece, Yugoslavia, Bulgaria, Turkey and southern Britain. It had also been eradicated in USSR, North America, several middle eastern countries, large parts of South America, the Caribbean, Australia, Japan and Taiwan. See the map.
Malaria Control through Habitat Control of Anopheles Mosquito Populations Led to a Dramatic Reduction in Prevalence of Malaria WorldWide

In India, the estimated number of malaria cases annually was estimated to be 75 million in 1950. This had been reduced to approximately 500,000 by 1970.

Drugs which can be used to treat malaria
Drug resistance has become a major problem in treating malaria. Thailand's experience shows a decrease in cure rates for drugs like mefloquine and chloroquine. Malaria deaths in ANE (2001) were 1.1 million.

Drugs and potential targets for new drugs against malaria include:

- **Cytoxon**: Drugs like antitoxin are targeted.
- **Glycolysis (e.g., pyruvate dehydrogenase)**: New targets include glycolysis and nucleotide biosynthesis.
- **Anaplast**: Drugs like antibiotics inhibiting protein translation (e.g., tetracycline and clindamycin) are considered.
- **Cytoplasmic Antigens**: New targets include ribosomal RNA replication and transcription.
- **Type 2 fatty acid biosynthesis**: New targets include fatty acid metabolism.
- **Mitochondrion**: Drugs like atovaquone inhibit cytochrome c reductase.
- **Plasmodium apicoplast protease**: New targets include apicoplast protease.
- **Glucose transport**: New targets include glucose transport.
- **Nutrient uptake**: New targets include nutrient uptake.
- **Na+/H+ antiport**: New targets include Na+/H+ antiport.
Loss of Function Mutations which Can Protect Against Malaria

- Duffy locus encodes the receptor for merozoites of *P. vivax* on red blood cells. Populations in West Africa with high incidence of *P. vivax* have a high frequency of loss of function allele at the Duffy locus.

- G6PD locus encodes a red cell enzyme which protects the red cell against reactive oxygen species produced by degradation products of hemoglobin; loss of function mutations for G6PD cause the malaria infected red cell to have a high level of the reactive oxygen species which are toxic to the plasmodium; G6PD loss of function alleles have high frequency in populations at high risk for malaria.
Balanced Polymorphism

Malaria
AA

Thalassemia
aa

Demonstrating the protective effect of red cell mutations against p. falciparum infection

Table 1. The observed malaria protective effect of common erythrocyte variants.

<table>
<thead>
<tr>
<th></th>
<th>In vitro</th>
<th>In vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection/multiplication (low O₂, conditions)</td>
<td>↓</td>
<td>↑</td>
<td>Protection: severe malaria (69–90%), mortality (55%)</td>
</tr>
<tr>
<td>HbAC</td>
<td>normal</td>
<td>N/A</td>
<td>Reduced risk: clinical malaria (59%), severe malaria (47–67%)</td>
</tr>
<tr>
<td>HbCG</td>
<td>↓ multiplication; altered knob formation</td>
<td>↑ susceptibility to phagocytosis</td>
<td>Reduced risk of clinical malaria (59%)</td>
</tr>
<tr>
<td>HbE</td>
<td>↓ parasite invasion (87%)</td>
<td>↑ susceptibility to phagocytosis</td>
<td>Reduced risk of complications (59% reduced odds)</td>
</tr>
<tr>
<td>HbEE</td>
<td>↓ invasion/multiplication</td>
<td>↑ susceptibility to phagocytosis</td>
<td>N/A</td>
</tr>
<tr>
<td>α-Thalassemia</td>
<td>Normal invasion</td>
<td>No difference from controls</td>
<td>Reduced risk of severe malaria (risk = 0.4–0.66)</td>
</tr>
<tr>
<td>β-Thalassemia</td>
<td>Inconclusive</td>
<td>↑ susceptibility to phagocytosis</td>
<td>Protection against hospital admission with malaria (59%)</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>↓ growth under oxidative stress</td>
<td>↑ susceptibility to phagocytosis (troph stage)</td>
<td>Protection of F(1) against non-severe malaria</td>
</tr>
<tr>
<td>PK deficiency</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

I, decrease; T, increase; M, male; F, female.
Hardy-Weinberg Equilibrium

- large population
- no mutation
- no selection
- random mating
- no migration

\[ [A] = p \]
\[ [a] = q \]
\[ p + q = 1 \]

\[ [AA] = p^2 \]
\[ [Aa] = 2pq \]
\[ [aa] = q^2 \]

frequencies remain stable

Hardy-Weinberg

<table>
<thead>
<tr>
<th>sperm</th>
<th>eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>allele</td>
<td>frequency</td>
</tr>
<tr>
<td>( A = p )</td>
<td>( AA )</td>
</tr>
<tr>
<td>( p^2 )</td>
<td>( 2pq )</td>
</tr>
<tr>
<td>( a = q )</td>
<td>( aa )</td>
</tr>
<tr>
<td>( pq )</td>
<td>( p^2 )</td>
</tr>
</tbody>
</table>

allele frequency
The only reason that Hardy-Weinberg should not hold at conception is assortative mating.
Selection: Genetic Lethal Can Eliminate One Genetic Class at Some Point in Life

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>Aa</th>
<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At conception</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>$q^2$</td>
</tr>
<tr>
<td><strong>After lethal events</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>0</td>
</tr>
</tbody>
</table>

Selection: Genetic Lethal Can Eliminate One Genetic Class at Some Point in Life

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
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<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At conception</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>$q^2$</td>
</tr>
<tr>
<td><strong>Early in life—some lethal events reduce aa class</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>Less than $q^2$</td>
</tr>
<tr>
<td><strong>Later in life—more lethal events reduce aa class</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>Much less than $q^2$</td>
</tr>
<tr>
<td><strong>After all lethal events</strong></td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>0</td>
</tr>
</tbody>
</table>
What matters for the next generation is the proportion of individuals in each genotype class at the time of mating and their relative effectiveness in mating—REPRODUCTIVE FITNESS

<table>
<thead>
<tr>
<th>generation</th>
<th>p</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.66</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Fitness

fitness: proportion of offspring compared with “normal”

coefficient of selection = 1-F

F = 1, s = 0 if normal number of offspring
F = 0, s = 1 if lethal
Change in Allele Frequency Because of Reduced Reproductive Fitness

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>Aa</th>
<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>$q^2$</td>
</tr>
<tr>
<td>Fitness</td>
<td>1</td>
<td>1</td>
<td>1-s</td>
</tr>
<tr>
<td>In Next Generation</td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>$q^2(1-s)$</td>
</tr>
</tbody>
</table>

Even after many generations, the recessive allele is still present at a significant allele frequency despite strong selective pressure.
Even though selection may remove all homozygous recessives from the reproductive pool, heterozygote matings recreate this genotype class in the next generation.

<table>
<thead>
<tr>
<th>Generation</th>
<th>P</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>2</td>
<td>0.66</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Why do different human populations have different allele frequencies for many genetic loci?

- Genetic drift
- Founder effects
- Mutation
- Selection
Genetic Drift

- fluctuation in gene frequency due to small size of breeding population
- fixation or extinction of allele possible
Founder Effect

- high frequency of gene in distinct population
- introduction at time when population is small
- continued relatively high frequency due to population being "closed"

![Diagram showing the founder effect process](image)

- Initial population
- "Bottleneck" where new population is derived from small sample
- New population with high frequency of mutant allele
When Two Populations Mix--How Long Does It Take To Reach Equilibrium if all Hardy-Weinberg conditions are met?

Population 1
- all AA

Population 2
- all aa

\[ [AA] = x \]
\[ [aa] = y \]
\[ x + y = 1 \]

<table>
<thead>
<tr>
<th>Mating Type</th>
<th>Frequency</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA x AA</td>
<td>(x^2)</td>
<td>All AA</td>
</tr>
<tr>
<td>AA x aa</td>
<td>(2xy)</td>
<td>All Aa</td>
</tr>
<tr>
<td>aa x aa</td>
<td>(y^2)</td>
<td>All aa</td>
</tr>
</tbody>
</table>

\[ [AA] = x^2 = p^2 \]
\[ [Aa] = 2xy = 2pq \]
\[ [aa] = y^2 = q^2 \]

Equilibrium in achieved in one generation for an autosomal trait
Hemoglobinopathies and Thalassemias

- Mutations which alter the function of either the alpha or beta globin genes
- Hemoglobinopathies—mutations which cause a change in primary structure of one of the globin chains—over 700 known
- Thalassemias—mutations which alter the level of expression of one of the globin chains—over 280 known

Figure 2 | The global distribution of the β-thalassemia mutations. The common mild mutations are shown in bold. β-thalassemia also occurs in the regions shaded in grey, but little is known about its molecular pathology in these areas.
Globin Chain Synthesis

- $\alpha_2^2\epsilon_0$ embryonic hemoglobin
- $\alpha_2^2\gamma_2$ fetal hemoglobin
- $\alpha_2^2\beta_2$ hemoglobin A
- $\alpha_2^2\delta_2$ hemoglobin A2
PATHOLOGY IN THALASSEMIA IS A CONSEQUENCE OF AN IMBALANCE IN ALPHA AND BETA GLOBIN CHAIN SYNTHESIS

EXCESS ALPHA OR BETA CHAINS ARE INSOLUBLE IN THE RBC

PRECIPITATED GLOBIN GENES DAMAGE THE RED CELL MEMBRANE SHORTENING RED CELL HALF LIFE

ANEMIA CAN BE CORRECTED BY TRANSFUSION

CONTINUOUS TRANSFUSION CAN LEAD TO IRON OVERLOAD
• Thalassemia peripheral smear
  Hypochromic
  Microcytic
  Target cells
  Variation in cell shape (poikilocytosis)
  Nucleated RBCs

Thalassemic bony changes

so-called thalassemic facies – “Completely avoidable” with modern hypertransfusion Rx
Thalassemia clinical features

Untreated thalassemia:
- growth failure,
- hepatosplenomegaly,
- severe anemia,
- severe bony changes,
- pathological fractures,
- iron overload by absorption

Heart, liver and endocrine disease

**Table: Thalassemia Genotypes and Syndromes**

<table>
<thead>
<tr>
<th>Alpha Thalassemia</th>
<th>α genes</th>
<th>Globin Chains</th>
<th>Hemoglobin</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>αα/ααα</td>
<td>α_2β_2</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Silent Carrier</td>
<td>αα/αα-</td>
<td>α_2β_2</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Trait</td>
<td>αα-/-αα</td>
<td>α_2β_2</td>
<td>A</td>
<td>Mild</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>--/-αα</td>
<td>α_2β_2, β_4</td>
<td>A, H</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>--/--α</td>
<td>γ_4, β_2γ_2</td>
<td>Bans Portland</td>
<td>Lethal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beta Thalassemia</th>
<th>β genes</th>
<th>Globin Chains</th>
<th>Hemoglobin</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>β/β</td>
<td>α_2β_2</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>β^+β/β</td>
<td>α_2β_2, α_2β_2, α_2γ_2</td>
<td>A, A_2, F</td>
<td>Mild</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>β^+β/β</td>
<td>α_2β_2, α_2β_2, α_2γ_2</td>
<td>A, A_2, F</td>
<td>Severe</td>
</tr>
<tr>
<td>HPPH+</td>
<td>γ/γ</td>
<td>α_2γ_2</td>
<td>F</td>
<td>Mild</td>
</tr>
</tbody>
</table>
THALASSEMIAS

- MUTATIONS HAVE BEEN IDENTIFIED IN ALPHA AND BETA GLOBIN GENES IN THALASSEmia WHICH AFFECT ALMOST EVERY PROCESS SIGNIFICANT IN GENE EXPRESSION

β-Globin Mutations Which Can Cause β-Thalassemia can occur throughout the gene
Splice variants are the most common causes of thalassemia alleles:

Consensus sequences around 5’ and 3’ splice sites in vertebrate pre-mRNAs. The only nearly invariant bases are the (5’ GU and 3’ AG of the intron, although the flanking bases indicated are found at frequencies higher than expected based on a random distribution. A pyrimidine-rich region (light blue) near the 3’ end of the intron is found in most cases. The branch-point adenosine, also invariant, usually is 20 – 50 bases from the 3’ splice site. The central region of the intron, which may range from 40 bases to 50 kilobases in length, generally is unnecessary for splicing to occur. (from Lodish et.al.-Molecular Cell Biology)
A Mutation in an Exon Can Create a New Splice Site Causing a Non-functional mRNA to be Made
Mutations in the Promoter, the 3’ UTR or the poly A Site Can Reduce mRNA Expression Levels

Eukaryotic promoters are organized in a modular manner
The TATA box is an important sequence for most eukaryotic promoters because it binds the key transcription factor TBP.

β Thalassemia mutations in the TATA box include:
- -31 A to G
- -30 T to A and -30 T to C
- -29 A to G
- -28 A to G

An important upstream element is located between positions -86 and -90 of the β globin gene.

β Thalassemia mutations in this element include:
- -90 C to T
- -88 C to A or T
- -87 C to A or G or T
- -86 C to G
Mutations affecting mRNA polyadenylation at the polyA site can cause β thalassemia

- AATAAAA is β globin poly A site
- Mutations seen in β thalassemia
  - AACAAAA
  - AATTAA
  - AATTGA
  - AATAAC

Capping the 5’End.
Caps at the 5’end of eukaryotic mRNA include 7-methylguanylate (red) attached by a triphosphate linkage to the ribose at the 5’end. None of the riboses are methylated in cap 0, one is methylated in cap 1, and both are methylated in cap 2.

Mutation in silent β thalassemia +1 A to C
A Mutation in the Chain termination Codon Causes Instability of the mRNA Leading to Reduced levels of Gene Expression

<table>
<thead>
<tr>
<th>mRNA Levels</th>
<th>Globin Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA Levels</td>
<td>h</td>
</tr>
<tr>
<td>α-Globin mRNA</td>
<td>UAA</td>
</tr>
<tr>
<td>αG mRNA</td>
<td>UAA</td>
</tr>
</tbody>
</table>

α thalassemia syndromes

- **Normal**
- **α-thalassemia genotypes**
- **α-thalassemia with Hb Constant Spring**
  - Heterozygous Hb CS (resembles α-Thal2)
  - Homozygous Hb CS (more severe than α-Thal1)
  - Hb H disease with Hb CS
  - Hydrops fetalis not observed with αG

- **α-Thal2 trait** (*Mild* trait, silent carrier)
- **α-Thal1 trait** (*Severe* α-Thal trait)
- **Hemoglobin H disease**
- **Hb Bart's hydrops fetalis**
LEVELS OF ALPHA AND BETA GLOBIN CHAIN SYNTHESIS MUST BE EXTREMELY HIGH BUT CORRECTLY REGULATED DURING RED CELL DIFFERENTIATION

AMOUNTS OF ALPHA CHAINS MUST BE APPROXIMATELY EQUAL TO AMOUNTS OF BETA CHAINS

REGULATION IS COMPLEX—DNA SEQUENCES TENS OF KBP AWAY FROM CODING REGION PLAY IMPORTANT REGULATORY ROLE

DNA sequences such as β LCR and HS40 play a Key Role in Controlling Expression of the Locus
Deletions which entirely eliminate the Beta Globin Gene Cause the Gamma Chain Genes to Remain On; Hereditary Persistence of Fetal Hemoglobin (HPFH)
Possible Strategies for Effective Medical Intervention in Thalassemia and Hemoglobinopathies

- Population screening
- Splenectomy and increased vigilance for infectious disease
- Transfusion accompanied by iron chelation therapy
- Drug treatment to increase fetal hemoglobin levels
- Bone marrow transplantation
- Gene therapy

Sardinian mutation $\beta^{\text{gln39x}}$
Genetic Screening in Sardinia

- High prevalence of β thalassemia heterozygotes in the population
- Aggressive carrier screening begun in late 1970s to allow reproductive choices
- Carriers detected by simple hemoglobin electrophoresis test which detects elevated levels of hemoglobin A2, a consequence of elevated δ chain synthesis
- Frequency of β thalassemia births reduced to very low levels

Current therapy (1)

- The mainstay of thalassemia treatment is transfusion to lowest ("trough") hemoglobin >9-10 g/dl: ‘Hypertransfusion’
- Goal: shut off endogenous erythropoiesis, or else complications of ineffective erythropoiesis will persist despite tx.
- Over time, nobody gets more fresh red cell transfusions than a thalassemia major patient.
- Consequent toxicity of iron poisoning
Iron and risk of complications

Hepatic iron content >15 mg/g dry wt as a risk factor for morbidity and mortality

Thalassemia and iron overload. Why is the iron toxic, and for whom?

- **Thal Major**
  - >8 transfusions/year
  - Trough Hb >9 g/dl shuts off erythropoiesis
  - High non-transferrin bound iron (NTBI) due to transfusion

- **End-organ iron damage**
  - heart, pancreas, pituitary, liver

- **Lifelong Chelation**
  - Potential DFO toxicity: retina, hearing, pulmonary, renal

- **Thal intermedia**
  - Survive without transfusion
  - Brisk, but ineffective erythropoiesis through life; massive RBC turnover
  - High NTBI from increased gut absorption and RBC turnover

- **End-organ iron damage**
  - similar but later than thal major

- **Thrombosis risk, shortened life-expectancy in poorly managed patients**
Current therapy

Deferoxamine infusion

- 10 hours a night, 5-7 days per week
- Subcutaneous admin.
- Pumps can be unwieldy, and infusions uncomfortable (newest pumps better)
- Dosages 30-50 mg/kg/day lifelong (kilograms)
- Tens of thousands of dollars per year.
- Reactions fairly common

Current therapy (2)

- Stem cell transplantation
  - Curative
  - Chance to remove iron by subsequent phlebotomy instead of chelation
- Limitations
  - Risk of up-front mortality
  - Risk of GVHD
  - Only 25% of full sibs will be HLA matched
  - Unrelated matched transplants less safe
  - Non-myeloablative transplants not proven successful
  - North American centers have transplanted fewer patients among those eligible, compared to European centers. Are we too conservative?
Bone Marrow Transplantation

- Use cytotoxic drugs to destroy the patient’s own hematopoietic stem cells
- Inject hematopoietic stem cells from the bone marrow of an HLA matched donor and allow stem cells to repopulate the hematopoietic system of the patient
HLA A, B and DR are the three loci in the MHC complex which are matched for a bone marrow transplant.

Polymorphism:

Polymorphism: occurrence of at least two alleles at a locus having a frequency of at least 1%
Haplotype

- A set of closely linked alleles (genes or DNA polymorphisms) inherited as a unit.
- A contraction of the phrase "haploid genotype".
- A specific combination of alleles at several closely linked polymorphic loci can be referred to as a haplotype.

The MHC locus is several megabases long; there is therefore very little recombination between HLA A, HLA B and HLA DR. So HLA genotypes are usually inherited as a haplotype.

The odds of two children matching haplotypes inherited from both parents and therefore being a suitable bone marrow donor for their sibling is 1 in 4.
Survival of children given bone marrow transplants for thalassemia from relatives with matched HLA genotype

Children in class 1 had no risk factors; class 2 had one of 3 risk factors: liver fibrosis, or hepatomegaly greater than 2 cm or irregular iron chelation therapy

What are the chances of having an HLA matched donor?

- A sibling has a 1/4 chance of being an HLA match
- Because the HLA locus is highly polymorphic the odds of another person in the population unrelated to the patient having an HLA match are small (1 in several thousand) or less
- If the patient is a member of a specific ethnic group, the most likely match is with a member of that ethnic group
- If the patient’s parents come from two different ethnic groups then the odds of a match are decreased still further
Survival of children given bone marrow transplants for thalassemia from unrelated donors with matched HLA genotype

Gene therapy in 2006

- Evidence this can work
  - Murine models with lentiviral vectors driving normal beta globin gene.
- State of the art, 2006:
  - Not ready for prime time in humans
  - Starting to work in mice
- Cautions:
  - Insertional mutagenesis with retroviral vectors
  - Magnitude of expression
  - Duration of expression
Thalassemia therapy-experimental

- Increased fetal Hb (γ globin) synthesis in beta thalassemia.
  - Evidence this can work
    - HPFH syndromes
    - Butyrate analogs
    - Chemotherapy agents
      - Nucleoside analogs
      - Hydroxyurea
  - State of the art 2006 – clinical trials in thal intermedia – many patients respond, but often not enough to be useful clinically.