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
Genetic Drift

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

Founder Effect
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

\([\text{AA}] = x\]
\([\text{aa}] = y\]
\(x + y = 1\)
### 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

---

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

- α: embryonic hemoglobin
- α₂γ: fetal hemoglobin
- α₂β: hemoglobin A
- α₂δ: hemoglobin A2

Globin Gene Organization

- α: embryonic hemoglobin
- α: fetal hemoglobin
- α: hemoglobin A
- α: hemoglobin A2

Alpha cluster on chromosome 16

Beta cluster on chromosome 11
THALASSEMIAS

- 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 blood smear--abnormal rbc properties
- Hypochromic (don’t stain as strongly because there is too little hemoglobin)
- Microcytic (cells are small because there is too little hemoglobin)
- Target cells (red cell with increased surface area to volume ratio--appear as targets with a bullseye)
- Variation in cell shape--polikilocytosis (a full amount of hemoglobin is necessary to maintain the shape of the rbc)
- Nucleated RBCs (anemia is so severe that cells are released prematurely from the bone marrow before the nuclei are removed in an attempt to produce an adequate number of rbc)

NORMAL BLOOD SMEAR  BETA THALASSEMIA BLOOD SMEAR
Thalassemia changes in the bones

Because of the increase in the amount of bone marrow, the bones are weaker and fractures such as this spinal compression fracture become more common.

The bones of the skull as well as all other bones in the body are expanded because of the increase in the volume of bone marrow attempting to make adequate numbers of red blood cells.

Untreated thalassemia

Severe anemia--red blood cell lifetime reduced from 120 days to one week or a few days

Growth failure because of anemia

Splenomegaly--The fine capillaries of the spleen are normally responsible for removing damaged red blood cells from circulation; the massive numbers of damaged red blood cells fill the spleen causing it to enlarge and eventually rupture catastrophically

Severe bony changes, pathological fractures,

Iron overload by absorption

Heart, liver and endocrine disease
### Thalassemia Genotypes and Syndromes

<table>
<thead>
<tr>
<th>Alpha Thalassemia</th>
<th>( \alpha ) genes</th>
<th>Globin Chains</th>
<th>Hemoglobin</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/</td>
<td>( \alpha\alpha/\alpha\alpha )</td>
<td>( \alpha_2\beta_2 )</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Silent Carrier</td>
<td>( \alpha\alpha/\alpha- )</td>
<td>( \alpha_2\beta_2 )</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Trait</td>
<td>( \alpha-/\alpha- )</td>
<td>( \alpha_2\beta_2 )</td>
<td>A</td>
<td>Mild</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>( -/-\alpha )</td>
<td>( \alpha_2\beta_2 ; \beta_4 )</td>
<td>A, H</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Hydrosps fetalis</td>
<td>( -/-\alpha )</td>
<td>( \gamma_4; ; \zeta_2 \gamma_2 )</td>
<td>Barts Portland</td>
<td>Lethal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beta Thalassemia</th>
<th>( \beta ) genes</th>
<th>Globin Chains</th>
<th>Hemoglobin</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/</td>
<td>( \beta/\beta )</td>
<td>( \beta_2\gamma_2 )</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>( \beta^+\beta/ \beta^{0}\beta )</td>
<td>( \alpha_2\beta_2 ; \alpha_2\gamma_2 )</td>
<td>A, A_2, F</td>
<td>Mild</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>( \beta^+\beta/ \beta^{0}\beta )</td>
<td>( \alpha_2\beta_2 ; \alpha_2\gamma_2 )</td>
<td>A, A_2, F, F, ( \beta_2 )</td>
<td>Severe Severe</td>
</tr>
<tr>
<td>HPPH*</td>
<td>( \gamma/\gamma )</td>
<td>( \alpha_2\gamma_2 )</td>
<td>F</td>
<td>Mild</td>
</tr>
</tbody>
</table>

**THALASSEMIAS**

- MUTATIONS HAVE BEEN IDENTIFIED IN ALPHA AND BETA GLOBIN GENES IN THALASSEMSIA 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

Mutation from G to A in the intron 1 creates an alternative splice acceptor
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.

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
  - AATTTA
  - 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
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

DELETION OF THE HS40 BOX LEADS TO INACTIVATION OF TRANSCRIPTION OF THE α GENE

Figure 6-4. Deletions upstream from the α globin genes that inactivate the α globin gene complex by removing HS40. Original data provided in reference 12. (Prepared by Dr. D. R. Higgins)
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 $\beta$ 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 $\delta$ chain synthesis
- Frequency of $\beta$ thalassemia births reduced to very low levels
Current therapy

- 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

![Graph showing hepatic iron content and risk of complications.](image-url)
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

- **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

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:
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.
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
Bubonic Plague in 19th and early 20th centuries

- Mortality rate remains 60% of infected patients until antibiotics are developed
- 1855 plague moves into China following military expedition across Salween River
- 1855-1894 Plague moves through interior of China
- 1894--Plague reaches Canton and Hong Kong; European bacteriologists come to Hong Kong
- Alexander Yersin isolates Yersenia Pestis, the bacteria causing plague

Bubonic Plague early 20th century

- Movement by steamship around world now possible
- Quarantines imposed limit plague at points of entry
- 1898 Plague breaks through quarantine in India, 6 million die before epidemic is brought under control
- 1911 and 1921 in Manchuria--plague moves back into population in Manchuria as population naïve to protective folk traditions is allowed to trap marmots infected with plague
- Plague travels by rail to Harbin but is contained by quarantine
Bubonic Plague mid 20th century

• 1940s--Plague becomes curable if correctly diagnosed at an early stage with antibiotics
Figure 1 | World distribution of plague, 1990. Modified from BEBS © (1990) National Academy of Sciences.
Figure 1: Steps in the transmission of the pathogenic yersiniae in humans. Y. pestis has a
recent ancestor. The rodent's flea acquires Y. pestis from a host that carries blood, and transmits
the bacteria primarily to other rodents, or occasionally to humans — causing bubonic plague.
Humans-to-human transmission can occur through human fleas. Pneumonic plague is
transmitted from human to human through respiratory droplets, or possibly by artificially
generated Y. pestis aerosols. Y. enterocolitica and Y. pseudotuberculosis are ingested, and in
contrast to Y. pestis, enter the lymphatic system through the M cells of the small intestine.
Table 1: Plasmids important in the virulence of pathogenic yersiniae

<table>
<thead>
<tr>
<th>Plasmid name</th>
<th>Other designations</th>
<th>Size (ko)</th>
<th>Virulence determinants</th>
<th>Role in disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yersinia virulence plasmid, pV</td>
<td>pDCD</td>
<td>70.8</td>
<td>Several YOPS, type III secretion system</td>
<td>Avoidance of the immune system, toxity</td>
</tr>
<tr>
<td>Plasmid-encoding murine toxin, pMT1</td>
<td>pMT1</td>
<td>9.0</td>
<td>Murine toxin (proteasease). F1 capsule-like antigen</td>
<td>Bacterial transmission by nasal</td>
</tr>
<tr>
<td>Plasmid-activating plasmid, pIPS</td>
<td>pPSP1, pPSP2</td>
<td>9.0</td>
<td>Plasminogen activator</td>
<td>Dissemination from intra-dermal site of infection</td>
</tr>
</tbody>
</table>
PATHOGENIC E. COLI

- ENTEROTOXIGENIC (ETEC) (secretory diarrhea)
- ENTEROPATHOGENIC (Malabsorptive diarrhea) (EPEC)
- ENTEROHEMORAGGHIC (Malabsorptive diarrhea and dysentery) (EHEC) (E. coli 0157)

All three types are major causes of infant deaths worldwide
Bacterial Toxins

- Endotoxins - bacterial lipopolysaccharides
- Exotoxins - specific polypeptides produced by bacteria which cause toxic effects

BACTERIAL EXOTOXIN GENES ARE OFTEN ACQUIRED BY GENE TRANSFER

*Figure 9-1* Lysogenic bacteriophages carry toxin genes that integrate into the bacterial chromosome, thus making the bacterium capable of producing the toxin.
MECHANISM OF ACTION ST AND LT TOXINS IN ETEC

INTERFERENCE WITH cAMP or cGMP METABOLISM LEADS TO LOSS OF CONTROL OVER WATER FLOW AND INTESTINAL WATER LOSS
DELIVERY OF EPEC TOXIC PROTEIN TO HUMAN INTESTINAL CELLS BY A TYPE III SECRETION SYSTEM

EPEC PILI BIND TO INTESTINAL EPITHELIAL CELLS; CHANNEL IS FORMED MAKING DIRECT CONNECTION BETWEEN EPEC CYTOPLASM AND INTESTINAL CELL CYTOPLASM TOXIC PROTEIN INJECTED WITHOUT EXPOSURE TO HUMAN IMMUNE SYSTEM

EHEC

- Shiga like toxin circulates through bloodstream, enters kidney and causes kidney damage
Control of Infectious Disease

- Sanitation and other environmental controls
- Innate defenses of the body
- Vaccination
- Anti-infective therapy

Sanitation as a Defense Against Infectious Disease

- Once germ theory of disease was understood --sanitary engineering became a key line of defense against infectious disease
Goals of Sanitary Engineering

• Remove wastes safely from environment
• Eliminate possible sources of contamination by infectious agents
• Provide safe water, food and air
What makes a microorganism or virus a significant human pathogen?

- Ability to effectively cause infection i.e. genes expressed which facilitate entry into body
- Ability to effectively replicate in the body and evade immune response
- Ability to effectively exit body in a form which can be transmitted directly or indirectly to a new human host
- Ability to produce gene products which cause pathological effects such as toxins and/or superantigens

Viral Gastroenteritis

- It is thought that viruses are responsible for up to 3/4 of all infective diarrhoeas.
- Viral gastroenteritis is the second most common viral illness after upper respiratory tract infection.
- In developing countries, viral gastroenteritis is a major killer of infants who are undernourished. Rotaviruses are responsible for half a million deaths a year.
- Many different types of viruses are found in the gut but only some are associated with gastroenteritis.
Rotavirus Particle

Rotaviruses

- Naked double stranded RNA viruses, 80 nm in diameter.
- Also found in other mammals and birds, causing diarrhoea.
- Account for 50-80% of all cases of viral gastroenteritis.
- Usually endemic, but responsible for occasional outbreaks.
- Causes disease in all age groups but most severe symptoms in neonates and young children.
- Asymptomatic infections common in adults and older children. Symptomatic infections again common in people over 60.
- Up to 30% mortality rate in malnourished children, responsible for up to half a million deaths per year.
Rotaviruses

- 80% of the population have antibody against rotavirus by the age of 3.
- More frequent during the winter.
- Faecal-oral spread.
- 24-48 hr incubation period followed by an abrupt onset of vomiting and diarrhoea, a low grade fever may be present.
- Diagnosed by electron microscopy or by the detection of rotavirus antigens in faeces by ELISA or other assays.
- Live attenuated vaccines under testing for use in children.

Adenovirus Particle

(Courtesy of Linda Stannard, University of Cape Town, S.A.)
Enteric Adenoviruses

- Naked DNA viruses, 75 nm in diameter.
- Fastidious enteric adenovirus types 40 and 41 are associated with gastroenteritis.
- Associated with cases of endemic gastroenteritis, usually in young children and neonates. Can cause occasional outbreaks.
- Possibly the second most common viral cause of gastroenteritis (7-15% of all endemic cases).
- Similar disease to rotaviruses
- Most people have antibodies against enteric adenoviruses by the age of three.
- Diagnosed by electron microscopy or by the detection of adenovirus antigens in faeces by ELISA or other assays.

Astrovirus Particles

(Source: ICTV database)
Astroviruses

- Small RNA viruses, named because of star-shaped surface morphology, 28 nm in diameter.
- Associated with cases of endemic gastroenteritis, usually in young children and neonates. Can cause occasional outbreaks.
- Responsible for up to 10% of cases of gastroenteritis.
- Similar disease to rota and adenoviruses.
- Most people have antibodies by the age of three.
- Diagnosed by electron microscopy only, often very difficult because of small size.

Calicivirus Particles

(Source: ICTV database)
Caliciviruses

- Small RNA viruses, characteristic surface morphology consisting of hollows, particles 35 nm in diameter.
- Associated mainly with epidemic outbreaks of gastroenteritis, although occasionally responsible for endemic cases.
- Like Norwalk type viruses, vomiting is the prominent feature of disease.
- Majority of children have antibodies against caliciviruses by the age of three.
- Diagnosed by electron microscopy only, often difficult to diagnose because of small size.

Norwalk-like Virus Particles

(Source: ICTV database)
Norwalk-like Viruses

- Small RNA viruses, with ragged surface, 35 nm in diameter, now classified as caliciviruses.
- Always associated with epidemic outbreaks of gastroenteritis, adults more commonly affected than children.
- Associated with consumption of shellfish and other contaminated foods. Aerosol spread possible as well as faecal-oral spread.
- Also named "winter vomiting disease", with vomiting being the prominent symptom, diarrhoea usually mild.
- Antibodies acquired later in life, in the US, only 50% of adults are seropositive by the age of 50.
- Diagnosis is made by electron microscopy and by PCR.