

Camb 550 Principles of Genetics Review Part III  
Human Genetics

## I. Cytogenetics

Cytogenetics is the visual analysis of DNA. It is used for clinical diagnosis involving constitutional abnormalities and cancer, as well as various research applications. It is based on the fact that chromosomes have a distinctive morphology, which is visible in their banding pattern. Cytogenetic abnormalities occur in about 50% of miscarried fetuses, as well as about 0.6% of live births.

The key technique for cytogenetics beyond simply examining the banding pattern is the use of FISH or Fluorescence in situ hybridization. Initially DNA is denatured on slides, and then labeled with a probe which has been fluorescently tagged. This probe is generally made homologous to the region being searched for. The DNA is rinsed to remove non-specifically bound label, and then counterstained, so that all chromosomes are visible. The results are then analyzed under a fluorescent microscope. Probed regions will be one color, while the remainder of the DNA will be another. In this manner specific losses and duplications can be visualized.

The other major technique is array comparative genomic hybridization (Array CGH). This is essentially a genome wide microarray version of fish. Shorter probes from various portions of the genome are attached to an array. In this manner the whole genome can be analyzed for losses or duplications.

There are numerous diseases which involve chromosomal loss or duplication, but they are probably not relevant here.

## II. X inactivation

In humans sex is determined based on the X chromosome. XX female, XY male. In most cases in women, one of these X chromosomes is inactivated in every cell. X inactivation occurs twice during early embryogenesis, the first is in the extra-embryonic lineage (placenta) in which the paternal X is inactivated. The second event is later in the embryonic lineage, and results in the mosaic seen in most females. The inactivated X stains darkly in somatic cells and is known as a Barr Body. The process of X inactivation is random.

The mechanism:

Counting/choice → Initiation → Propagation → Maintenance

Counting: Only one X chromosome is active in the diploid genome, all others are inactivated. Somehow the X to autosomal ratio is determined, the mechanism for this is not known. There are phenotypes associated with extra X chromosomes which are not inactivated.

Choice: There is some possibility that different genetic loci affect it. Different alleles have different strengths and are always inactivated over one or another (as in mouse). But basically we got no clue.

Initiation: Structural evidence suggests one location which initiates inactivation. The X inactivation center (Xic) coordinates differentiation and counting signals. X inactivation spreads in cis from the Xic. A gene called Xist, was identified in the Xic region and is exclusively expressed from the inactive X. Disruption of Xist results in an active X chromosome.

Propagation: Not all genes are inactivated on the inactivated X chromosome. The expression of these genes is variable across the population. Older, more ancestral portions tend to be inactivated. Inactivation seems to occur in domains, and is potentially mediated by L1 elements, chromatin and promoter strengths (or lack thereof).

Maintenance: Heterochromatin formation. DNA methylation, novel histones (macroH2A), late DNA replication and changes in histone modification serve to maintain the inactivation.

Rich's lectures:

\*\*\*\*\*

III. Principles of Population Genetics

IV. Analysis of Genetic Linkage in Humans

V. Complex Genetic Disorders

\*\*\*\*\*

These are covered in his handouts better than I could do. We will use these to study.

VI. Genomics (Given short shrift as it is covered elsewhere)

HapMap

SNPs and how they can be used as markers for disease. When they are close they tend to stay associated. Association studies can be performed to determine.

Microarrays: Oligonucleotide and cDNA. Genome wide expression profiling.

Chromatin Immunoprecipitation.

Array CGH

FISH

Tissue Microarrays (Tissue spotted rather than genes).

VII. Cancer Genetics

A swift review of cancer genetics

There are six key properties of cancer cells:

They disregard normal proliferative signals

They become resistant to apoptosis

Tend to escape senescence and avoid differentiation

Become genetically unstable

Develop the ability to invade normal tissue

Survive in the circulation and grow in inappropriate locations

Benign → Dysplasia (pre-cancerous phenotypic changes) → Carcinoma (small tumor)

→ Malignant (capable of invading surrounding tissue) → Metastases (2ndary tumors)

Evidence from X inactivation indicates that cancers have a monoclonal origin.

There are several types of cancer critical genes:

1. Proto-Oncogenes: Genes which if mutated confer a growth advantage.
2. Tumor-suppressor: Genes which normally repress growth.
3. Caretaker genes: Function to maintain genes or genomes

As always, Ras has the potential for cancer, no lecture would be complete without at least a half dozen ras slides, this is no exception.

p53 is a really critical gene, it is mutated in 60% of human tumors. Activation of p53 leads to growth arrest and apoptosis, for this reason cancers cells without it see a major growth advantage. Mutation of p53 is indicative of a generally poor prognosis.

VIII. Gene Diagnosis

Gene diagnosis is key for learning more about genetic diseases. It is useful for looking at the following things:

If a mutation is autosomal recessive or X-linked

Prenatal diagnosis

Autosomal diagnosis – pre-symptomatic

Clinical diagnosis

Direct diagnosis of disease is becoming increasingly possible; however, more complex diseases are more difficult. Diagnosis can be performed by linkage to DNA polymorphism, however, factors such as non-paternity and meiotic recombination between the marker and the disease make it difficult. Tests can be done prenatally by sampling the chorion villus (tissue surrounding the fetal gestation sac, 12 weeks pregnant) or by amniocentesis (16 weeks pregnant). The latter method having near negligible risk. A simple test is one involving a changed restriction site, such as with sickle cell anemia. Diseases such as CF are difficult due to the heterogeneity of mutations involved. Reverse Dot hybridization tests, use PCR and a probe to test for point mutations. These are useful for performing batteries of tests in search of potential mutations.

At what sensitivity/specificity is screening worthwhile?

There are numerous treatment strategies, which act at various stages of diseases.

Treatments can act on the mutant gene itself, the mutant RNA or the mutant protein. They can do this by supplementing, modifying or out-competing native mutant proteins.

In viral vectors for gene therapy several issues are important:

- 1) Safety
- 2) Easy to make
- 3) Easy to introduce to target tissue
- 4) Life long expression of gene of interest at correct levels

There are several potential vectors:

1)Retrovirus

Advantages: Enters every cell, rendered incapable of replication, non-toxic, only a few copies integrate, stable integration, 8 kb of DNA max

Disadvantages: Integrates only in dividing cells, integrated gene often shut off, high incidence of leukemia post integration

2)Adenovirus:

Advantages: Grows to high titer, infects a variety of cells dividing and non-dividing, large DNA inserts (30 kb)

Disadvantages: Expressed for only weeks, elicits strong immune response

3) Adeno-Associated Virus

Advantages: Widespread in humans with no ill effects, infects a variety of cells, exists either integrated or non-integrated, less immunogenic

Disadvantages: small, only accommodates 5 kb of DNA