HST.161 Molecular Biology and Genetics in Modern Medicine
Fall 2007

Lecture 1 Overview, Basics

This course connects to other courses you're taking; by figuring where in the "instruction manual" (genome) the error is, one can start working out what the problem is.

Goals of Medical Genetics:

- identify patterns of DNA seq. variation which contribute to or cause diseases
- use this knowledge to understand the underlying molecular basis of pathology
- use this knowledge to provide diagnostic insight and information to patients
- develop treatments and cures; preventative, or stop something in-progress or advanced

Case of a 48-year-old man with sudden loss of consciousness while jogging[1]. He had previously been well and had no idea that he carried a genetic mutation that caused his heart defect - hypertrophic cardiomyopathy. We'll discuss a similar case on Tuesday (hypertrophic cardiomyopathy).

The goal of this course is to give you enough knowledge of genetics and molecular biology that you learn how to critically read these types of reports and get something valuable out of them.

How do we get to the idea that something has a genetic basis?

- family studies; Mendelian inheritance patterns
- chromosomal analysis correlates clinical symptoms with chromosomal aberrations
- relative frequency of symptom patterns shared by genetically-related individuals is higher than in less related or unrelated individuals (a lot of effort is going into this method)
- specific DNA sequence differences observed in clones of somatic cells correlated with specific clinical phenotype (such as tumor cells)

Case study of a 23-day-old infant who had trouble feeding and a failure to thrive[2]; he was referred to Mass. General and it was found that he had a derivative chromosome due to a chromosomal inversion – he was missing a significant portion of one of his copies of Chromosome 16. Trisomy 16 is the most common cause of miscarriage, but missing part of a 16 was somewhat unique; it turns out that this same family had been seen before for another instance of missing a portion of the chromosome. A chromosome 16 inversion segregates in this family. The people who have the inversion are normal, but when the inverted 16 recombines during gametogenesis, the offspring can be abnormal.

[1] Binder, William D, Michael A. Fifer, Mary Etta King, and James R. Stone. "Case 26-2005: A 48-Year-Old Man with Suddent Loss of Consciousness while Jogging." *N Engl J Med* 353 (2005): 824-832.
[2] Stoler, Joan M., Natalia T. Leach, and Patricia K. Donahue. "Case 36-2004: A 23-Day-Old Infant with Hypospadias and Failure to Thrive." *N Engl J Med* 351 (2004): 2319-2326.

Case study of an 11-year-old girl with sudden loss of vision in her right eye[3]. She didn't have any pain or excess tears, so her parents waited to see what happened for about a month before seeing a medical professional. Her eye had turned white; they found a tumor in her eye, a retinoblastoma. Her eye had to be removed and her whole family tested for RB.

The rare things always happen to *someone;* these rare things can actually teach us about the more common things that happen.

Important note: the observation of a Mendelian inheritance pattern means that there is ONLY ONE site in the DNA responsible for the phenotype, in that family. If there was more than one gene/one site responsible, the inheritance pattern would be more complex.

- There are some people who claim that all sites responsible for phenotypes with Mendelian inheritance patterns have been cloned and are known – that's NOT TRUE.
- Some phenotypes can be caused by aberrations in the noncoding regions of DNA; we're currently trying to figure out exactly how this works. There are some syndromes with Mendelian inheritance patterns that have their roots in the noncoding DNA.

General information can be gained by chromosomal analysis due to repetitive aberrations in the genome (across many different people) that cause the same or similar clinical symptoms.

How do we identify the sites of genetic variation?

- single base pair differences (SNPs) and differences in small numbers of adjacent base pairs (frequency with which a particular site is changed varies dramatically)
- insertion or deletion of a DNA sequence including duplication and expansion or contraction in the numbers of copies of a repeated sequence
- rearrangements including inversion and translocation
- variation in chromosome number (doesn't show up as a variation in DNA sequence)
- epigenetic variation (also never shows up as a variation in sequence, because it is due to other kinds of markers on the DNA (such as methylation and imprinting))

Just a note: each chromosome is one large molecule, but in the lab, you'll only ever work with pieces of each.

[Review of DNA and RNA]

Physical properties of DNA: see slide

An important thing to note is that DNA must be packaged tightly in order to fit into the cell. The repulsive forces due to the negative charges on the outside of DNA, however,

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[3] Walton, David S., et al. "Case 5-2006: An 11-Year-Old Girl with Loss of Vision in the Right Eye." *N Engl J Med* 354 (2006): 741-748.

make this hard; this is why there are histones. The positive charge on the histones, around which DNA is coiled, neutralizes the negative charge and enables the DNA to be tightly wound around histones in a compact, beads-on-a-string type structure.

Variations and errors in DNA are what we're interested in.

WHEN the mistake happens matters!

- Mutation in a germ cell means it will be transferred to all cells of the zygote.
- Mutation in a somatic cell can lead to clones of that cell, containing the mutation.

There are checkpoints – quality control points – built into human DNA metabolism. Proofreading and error checking occurs, errors can either be fixed or the cell might have to self-destruct.

[Review of PCR and DNA sequencing]
When reading off sequence in the computerized method of fluorescent peaks, you need to be really careful to not miss any double peaks. Double peaks are due to heterozygosity; there are two strands of DNA, one from each copy of the chromosome.

Massively parallel sequencing technologies

- You "grow" many DNA molecules on a solid support; you don't get the same problem of missing double peaks, because you have more than one strand of DNA to look at (it's a larger signal, kind of)

Interspersed repetitive DNA sequences make many molecular biology procedures more difficult for mammalian genomes, such as Southern blotting, PCR, FISH, and comparative genomic hybridization

What is the frequency of human mutation?

- Point mutations: can occur as frequently as 1/20,000 bp per human generation, but generally occur at 1/10^8 bp per generation, at most sites, due to proofreading and error correction
- Unequal crossover: can occur as high as 1/7000 per human generation
- Anueploidy: can occur as often as 1 in 3 conceptions

Achondroplasia: autosomal dominant mutation in the FGFR3 gene; being homozygous for it causes death. (This is why there's a 2/3 chance of having it when both parents have it; the AA dies.)

- highly dependent on age of the father, since de novo mutations occur during cell division in male primary germ cells
- this mutation causes problems because it changes an important signaling ability
- mutation causes a glycine -> arginine change (a G turns into an A)
- a C followed by a G is the only place in your genome at which a methyl group can be placed on a C; this mutation then, is less likely to be repaired because demethylation of this C creates a thymine, which is a normal base, so it's not repaired

21-hydroxylase deficiency: important message: wind up reducing androgens when you shouldn't, which causes problems like not enough aldosterone, salt loss, etc. The problem is a pseudogene; it can either try to cross over with a good gene and kills it, or undergoes gene conversion which also kills the good gene. So, a dead gene next door can kill.

Duplicated sequences crossing over can cause loss of a large amount of a chromosome.

Muscular dystrophy [see slides]
We'll read a paper on this so don't have to worry about it for now.

[Review of Mendelian inheritance; genotype and phenotype.]

Penetrance: proportion of people with the genotype who actually express the phenotype
Age-dependent penetrance: penetrance as a function of age
Expressivity: severity of the phenotype

[review of Mendelian pedigree patterns]