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HST.161 Molecular Biology and Genetics in Modern Medicine  
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Lecture 4

Positional Cloning and Haplotypes; Cystic Fibrosis

***Cystic Fibrosis***

By Richard Parad

**Historical Background:**

- 300 years ago: descriptions of “bewitched” babies who had a salty taste when their mothers kissed them
- 1936-38: Pathologic descriptions of cystic pancreas associated with bronchiectasis (chronic inflammatory or degenerative condition of one or more bronchi or bronchioles - discovered upon autopsy of children who died). No way to diagnose prior to death, at this point.
- 1940’s: autosomal recessive; disorder of exocrine glands
- 1950s: abnormal sweat Cl<sup>-</sup> concentration; crude test, but is important diagnostic test
- 1980s: abnormal Cl<sup>-</sup> transport in epithelial cells and sweat glands; later, mapped to long arm of Chrom. 7
- 1989: trio of famous papers in *Science* described identification of gene (CFTR: cystic fibrosis transmembrane regulator)

**Cystic Fibrosis:**

- Most common autosomal recessive disorder in Caucasians, though it’s also present in other ethnicities
- Median age of survival is now 36, with improved care
- CFTR is part of the ATP binding cassette (ABC) superfamily of proteins
  - o Over 1500 mutations identified

**CF categorized in two forms:** classic and non-classic

Classic:

- no functional CFTR protein
- non-functioning pancreas; problems with fat digestion and malabsorption and lack of important pancreatic enzymes; leads to other diseases
- lungs develop severe infections and obstructions which ultimately destroy the lungs; this is what now kills modern CF patients
- liver problems
- male reproductive tract is affected – congenital absence of the vas deferens
- 20% of CF newborns are born with obstruction of the intestines
- Chronic sinus infections

Non-classic:

- some functional CFTR
- have fewer number of symptoms; some isolated problems rather than the full spectrum

Age Specific Prevalence in Respiratory Infections in CF Patients

- lungs become colonized by bacteria, which have become resistant to antibiotics; some can be lethal at a very young age
- we don't understand why having an abnormal CFTR protein enables these bacteria to grow in the lungs

**Pathophysiology:** Why are so many organs affected?

Common Threads:

- organs with tubular structures are particularly affected
- lined with secretory epithelial cells
- secretions are abnormally thick and dry due to too little water
- obstructed tubes lead to back pressure and injury of proximal tissue (airways, intestines, pancreatic ducts, hepatic ducts, vas deferens)
  - o patients with mildest functions have more problems in smaller tubes (e.g. vas deferens), while larger tubes are more forgiving

**Diagnosis:**

- History: respiratory, GI, infertility, FHx
- Pilocarpine iontophoresis (sweat test)
  - o Sweat [Cl<sup>-</sup>] determined by flame photometry
  - o (75 mg sweat) Normal: <40, Borderline: 40-59, CF: >60
- Genotype (less expensive than it used to be, but still pretty pricey; sweat test is much much less expensive)
- Other clinical: sputum cx, chest X-rays, stool fat, GU

Incidence of CF in live births in U.S.:

Caucasian: 1/2500

Hispanic: 1/9600

African-American: 1/17000-19000

Asians: 1/30000

Age at Diagnosis:

- tends to be diagnosed very early, depending on severity and typical/atypical type

Cellular disease mechanism:

- When CFTR is missing or nonfunctioning in the cell membrane, the outwardly rectifying Cl<sup>-</sup> channel closes, and the Na<sup>+</sup> channel opens more, allowing more sodium into the cell.
- There are different ways for this to go wrong; there can be problems with the protein itself, or with transporting the protein upon its creation to the cell membrane.
  - o Class I mutation: most severe: defective protein production
  - o Class II: defective processing
  - o Class III: defective regulation
  - o Class IV: defective conduction
  - o Class V: splice mutation causes diminished amount of protein to be made, but function is normal

[delta-F means mutation in protein; # after it (e.g. deltaF508) means protein # in protein chain]

Modifiers of disease severity:

- percent functional protein
  - o location of mutation
  - o amount of CFTR made
- associated cell membrane proteins
- other modifier genes (on other chromosomes) (currently being investigated)
- environment (especially relevant to problems in the lungs)

Infertile male patients (congenital bilateral absence of the vas deferens) were found to have only 5T in intron 8 (chrom 7); worst symptoms occur with both alleles being 5T, best (lack of symptoms) occurs with both copies having the full 9 Ts there. There's a spectrum in between of 5T/7T, 7T/7T, 7T/9T, 9T/9T.

Translating scientific knowledge into patient care:

- want to treat patient as early as possible

Treatments:

- gene therapy (get gene into progenitor cell, not epithelial cell; these cells are hard to target, though)
- gene "read through" therapy (reads over mutations)
- ion channel modulators
- anti-infectives
- anti-inflammatory
- lung transplants for severe respiratory problems
- most money is being put into trying to improve or increase protein function

Newborn screening for cystic fibrosis:

- until 1970's, test for immuno-reactive trypsinogen (IRT) done on a drop of blood; if levels were high, they would come back for a sweat test
- in the 1990's, began doing a combination test of DNA and IRT
  - o you don't want to rely on a DNA test alone because all the carriers for the disease will give you a lot of false positives
- early intervention, with nutritional therapies and early treatment of respiratory infections, helps a lot
- Tag-It™ genotyping system: tag PCR primers with small tag sequences, make the primers for specific mutations, or for WT. Labeled microspheres recognize the different tag sequences, and can be identified with lasers. This system allows you to tell whether someone has the mutation, and also tells you if someone is homozygous or heterozygous, as well.

Philosophical/ethical issues:

- Prenatal diagnosis is most important in early lethal disorders
- Prenatal (population screening) v. postnatal (newborn screening) testing