Module III: Evolution, diagnosis, and treatment

- See section manual for details
- Is there any evolutionary benefit to the mutant genetic element? Are there a variety of mutations or a conserved mutation? Why?
- Discuss the tests used to diagnose the disease. How do they work? How accurate are they?
- Discuss recent studies of cures, treatments, and prevention.
- What drugs are available or are in trials? How do they work? What is the cost?
- Suggest a treatment or cure of your own creation. Think about what steps in the biochemical pathway are good points of intervention and could be bypassed or altered to treat the disease.

Review--Cystic Fibrosis

- Cystic Fibrosis causes the body to secrete thick, sticky mucus in the pancreas, lungs and other exocrine glands.

- Mucus is caused by abnormal transport of chloride and other ions into and out of the cells.

- CF results from a missing/malfunctioning CFTR protein
  - transmembrane protein that forms a channel that regulates the flow of Chloride ions in and out of cells.

- Hundreds of reported CFTR mutations, but 70% of CF patients have a homozygous deletion of phenylalanine at codon 508
Cystic Fibrosis: Evolutionary Benefit

Is there any evolutionary benefit to the mutant genetic element? Are there a variety of mutations or a conserved mutation? Why?

- Heterozygotes may be more resistant to Typhoid fever, *Cholera*, and chloride ion-secreting diarrheas.
  - *Salmonella typhi*, which causes typhoid fever, uses CFTR for entry into epithelial cells. Heterozygous delta-F508 Cftr mice internalized 86% fewer S. typhi than did wildtype mice.
  - *Cholera* and pathogens that cause chloride ion-secreting diarrheas also use CFTR as a gate into the cells.
- Deletion of codon 508 (TTT--phenylalanine) accounts for 70% of mutations in CF patients
- Because the disorder is recessive, most mutations are inherited.


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**Diagnosis**

- The first clinical problem is during neonatal period in 10-20% of cases
  - intestinal obstruction from thick meconium—the first feces of the newborn (a condition known as *meconium ileus*)
- Other presentations are general nutritional deficiencies or recurrent bronchial infections
- The major clinical problems for CF are:
  - Pancreatic insufficiency
  - Recurrent pulmonary infections
Diagnostic tests

Don’t reiterate clinical symptoms from Module I, but instead focus on most often used tests. Also mention any molecular-based tests (PCR, mapping).

Most common clinical test is the Sweat Test - chloride content is measured.

• Elevated in CF patients (>60 mmol/L for children, >80 for adults)

• This test is extremely sensitive for cystic fibrosis and is the best test to diagnose CF

(Related age-old test: taste the forehead of child!)

Source: Cystic Fibrosis Foundation, OMIM, Medline

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Diagnostic tests

Genetic tests
• CF Carrier Test for potential parents with family history of CF
  • Blood sample or swab of cheek cells

• Prenatal tests
  • Amniocentesis--amniotic fluid sample containing fetal cells
  • CVS (Chorionic Villi Sampling)--chorionic tissue sample (cells derived from same lineage as fetus)

• These tests involve examining DNA for mutations and often use techniques of PCR and hybridization of DNA probes to detect different mutations.

www.genzymegenetics.com
Diagnostic tests

“Current testing readily detects up to 90% of carriers in the North European white population living in North America and 95% of the CF carriers in the Ashkenazi Jewish population. Approximately 50% of carriers in Hispanic and 75% of carriers in African American populations are detectable.” (OMIM)

• Why are there differences between different populations?
• Why are the tests not 100% accurate?

Cures and Treatments: The Pathway to New Therapies

• CF therapeutic development is very advanced relative to other diseases, and can be categorized into different types of therapies:
• Gene therapies: addition of good CFTR via adenovirus or other method
• Protein-assist and chloride channel therapies: treatment to help mutant CFTR get to cell surface and enhance function
• Anti-inflammatory and anti-infection therapies: for treatment of symptoms (inflammatory response and bacterial infections)
• Nutritional therapies: treatment to boost weight gain, pulmonary function, and overall health
• Many new therapies under development in clinical phases

Source: www.cff.org
Cures and Treatments: The Pathway to New Therapies--Drug Development Process

• Preclinical research--basic research in laboratories, in vitro and in animal models (1-5+ years)
• Clinical trials (in vivo) involve several phases over many years:
  • Phase I: determine safe doses and administration route (oral, IV, etc) (6-8+ months)
  • Phase II: Test drug for efficacy vs. a placebo (10-12+ months)
  • Phase III: Test a large number of people and fine tune the dose, ensure drug safety and effectiveness. (1.5+ years)
  • Phase IV: Examine long-term safety and effectiveness of the drug

Source: www.cff.org
Cures and Treatments

Gene therapies: delivery of good copy of CFTR gene to lung epithelia
Aerosolized adenovirus as delivery system
• Phase I demonstrated safety and tolerability
• Phase II studies failed to show sufficient improvement in lung function of CF patients.
• Study ended on 3/17/05
Compacted DNA as delivery system
• DNA passes through cell membrane and into nucleus with no other delivery mechanism
• Phase I completed--safe and tolerable

Protein-assist, Chloride Channel therapies: restore function to defective CFTR--one example:
• Phenylbutyrate--enables defective protein to localize to the cell surface
• Genistein--enhances activation of the chloride channel

More CF treatments

Anti-inflammatory and anti-infection therapies:
• Pulmozyme--DNAase that thins mucus in lungs
• Azithromycin--antibiotic for Pseudomonas infections
  – 50% reduction in hospitalizations, lung function improvement, and weight gain
• TOBI--tobramycin inhalation antibiotic for lung infections
• Ibuprofen and Immunitin--anti-inflammatories which can slow down degeneration of lung tissue

Nutritional Therapies:
• Digestive enzymes, vitamins--taken in pill form with meals
• New, more efficient pancreatic enzymes in Phase II trials (TheraCLEC-Total)

Other:
• 2000 Pseudomonas aeruginosa genome sequenced--the most common CF bacterial infection
  – Genomic analysis to provide info on how bacteria causes infection
  – Large potential for new therapeutics
Designing a treatment strategy or cure for your disease:

- Think about what you have learned about your disease’s molecular and biochemical mechanisms for causing disease.

- What steps in the biochemical pathway are good points of intervention to alter or bypass in order to treat the disease?

- Does the disease protein interact with proteins or other molecules?

- Drug companies often start with a protein of interest and find molecules (drugs) that bind to it and interfere with its action.

- Is gene therapy feasible for your disease?

- Just try your best!

Take Home Message

- Defective CFTR gene may have offered some populations a slight fitness advantage when present in heterozygous state, thereby causing its current uneven allelic distribution.

- The Sweat Test is commonly used to diagnose CF patients. Other tests exist and genetic testing can be performed prenatally or on potential parents.

- Many therapies exist for treatment of CF symptoms.

- Drugs have to go through at least 3 phases for FDA approval.

- Gene therapy cures are being very actively researched.