Integrating Pathophysiology and Genetics - Cystic Fibrosis
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Learning Objectives

• Describe the genetics of cystic fibrosis, the inheritance pattern, the protein that the gene encodes and the theory on how the genetic mutation leads to the clinical manifestations of the disease.
• List the organs involved in cystic fibrosis and the resultant clinical manifestations.
• Describe how you would diagnose a patient who you suspect may have cystic fibrosis.

Suggested Reading

• Robbins p 465-471 (Inborn Errors of Metabolism and Other Genetic Disorders)
**CF- History**

- **1700’s** - "Woe is the child kissed on the brow who tastes salty, for he is cursed and soon must die"
- **1905** - Meconium ileus first described
- **1938** - Dr. Dorothy Andersen
  - "Cystic Fibrosis of the Pancreas and its relation to celiac disease: a clinical and pathological study"
- **1940’s** - Autosomal recessive
- **1953** - Sweat electrolyte defect described during NYC heat wave
- **1959** - Sweat test first described
- **1983** - Sodium and Chloride transport abnormalities described
- **1989** - CFTR cloned
  - Positional cloning

**CF- Epidemiology**

- 1:2000 to 1:17,000 live births depending on the population.
  - 1:569 in Ohio Amish
  - 1:2,500 American whites
  - 1:17,000 in American blacks
  - 1:90,000 in Hawaiian Asians
- 1 in 25 North American whites are carriers
CF- Presentation

- Presenting features
  - Acute/persistent pulmonary symptoms- 51%
  - FTT/malnutrition- 43%
  - Steatorrhea- 35%
  - Meconium ileus- 19%
  - Family history- 17%
- Median age at diagnosis is 6 months
- 10% of diagnoses made after age 18.

CFTR

- Cystic Fibrosis Transmembrane Regulator
- Present in epithelial cells (pancreas, salivary glands, sweat glands, intestine, respiratory and reproductive tracts).
- Gene is on the long arm of chromosome 7, 250kB in length, 27 exons, 6.5 Kb mRNA
- Protein is 170 kD, 1480 amino acids
CFTR

- ATP Binding Cassette (ABC) Transport Family
- Protein Kinase A
- ATP regulated Chloride channel
- Regulatory protein for other channels
- Only 5-10% activity needed for normal chloride transport

Hypothesized Structure of CFTR

Cell 2016;167:1586–1597
CFTR Function

- Functions of CFTR are tissue specific (and so are abnormalities caused by the mutations).

Other Regulatory Roles of CFTR

- Epithelial Na Channel (ENaC)
- Electroneutral Na absorption
- HCO3/Cl exchanger (pancreas)
- ICOR Cl channel
- Ca and swelling activated CL channel
- ROM K2 K channel
- Gap junction channels
- Mucus secretion
- ATP transport
- Glutathione transport
- AQP3,7 (water channel)
- KCNN4 K channel
- KVLQT1 K channel

CFTR Mutations

- Cystic Fibrosis is an autosomal recessive disease.
- Currently >2000 mutations of the gene have been described
- Mutations are closely linked to infertility and pancreatic function, but not to pulmonary function
  - Exposures?
  - Disease modifying genes?

ΔF508 Mutation

- Most common mutation is a 3 base deletion at position 508, removing a phenylalanine (ΔF508)
- 88% of all CF patients have one copy of ΔF508, 50% are homozygous
- Frequency depends on ethnicity
  - In Ashkenazi Jews, W1282X is the most common mutation
Classes of Mutations

CFTR Mutations

- Despite 20+ years of research, it has been difficult to connect how the CFTR defect leads to the pulmonary disease.
- Why?
  - Difficult to set up in vitro model of epith cells
  - Variety of tissue specific roles of CFTR (too much focus on the sweat gland)
  - Transgenic mouse model does not have spontaneous lung disease
  - In vivo, difficult to measure Airway Surface Liquid (ASL) characteristics

Theories

- “ASL low volume” leads to mucus stasis, decreased ciliary beat, inhibited bacterial clearance.
- “ASL too salty” and salt sensitive defensins don’t function.
- Abnormal submucosal gland secretions.
- Abnormal modulation of epithelial inflammation
• **High salt model**: This is what happens in the sweat gland, chloride cannot be reabsorbed, sodium and water reabsorption are also decreased, ASL becomes very salty.

• **Low volume model**: Major defect is lack of regulation of ENaC. Na is hyperabsorbed, Cl follows through other pathways, water follows passively, low volume of ASL leads to increased mucus concentrations, delayed transport, mucus adhesions.
Mouse Model Supporting the Low Volume Model
• Airway specific over-expression of the epithelial sodium channel (ENaC or SCNN1) lead to ASL depletion, increased mucus concentration, delayed mucus transport, airway inflammation, poor bacterial clearance, early mortality


CF- Pulmonary Disease
• The lungs are normal at birth, but secretions and/or clearance is abnormal leading to inflammation, chronic infection, obstruction, bronchiolitis and bronchiectasis
• Symptoms- chronic productive cough, dyspnea, chest tightness
• Can have hemoptysis, pneumothorax as a complication
Pulmonary Pathology

CF- CXR

- Upper lobe predominant bronchiectasis
  - Signet ring
  - Tram tracking
- Peribronchial cuffing
- Nodules/mucous impaction
- Blebs, cysts
Respiratory Infections

Why Pseudomonas?

- No one really knows
- ASL milieu
- Cell receptor
- High salt concentrations/defensins
- Pseudomonas exhibits changes in chronic infection
  - Mucoidy, biofilm
  - LPS changes
  - Loss of flagella dependent motility
  - Slower growth
Respiratory Infections in CF

• Burkholderia cepacia (genovar III) is associated with rapid decline.
• Aspergillus fumigatus is commonly cultured (>50% of adults). Infection vs colonization?
• Emerging pathogens: Stenotrophomonas maltophilia, Achromobacter xylosoxidans, Mycobacterium abscessus

CF- Sinus effects

• Hypertrophy/hyperplasia of secretory elements
• Inflammation and edema
• Polyps
• Transepithelial electric potential is raised.

CF- GI tract effects

• Meconium ileus (5-15% newborns)
• Distal intestinal obstruction syndrome
• Rectal prolapse
• Focal biliary cirrhosis (25%)
• Hypoplastic GB (25%), gallstones (10%)
• Fatty Liver (30%)
GI effects of CF

- [Image]

[Link to image of a baby with meconium ileus]

http://pediatric.um-surgery.org/clinical/physician_content/a-m/meconium_ileus.shtml

CF- Pancreatic effects

- Obstruction of ducts with inspissated secretions leads to dilation, destruction and fibrosis.
- Exocrine insufficiency/fat malabsorption

[Image]

From the Geneva Foundation for medical research web site:

Pancreatic exocrine insufficiency

- Diagnosis
  - Symptoms
  - 72 hour fecal fat collection
  - Fecal chymotrypsin or fecal elastase
  - Vitamin ADEK levels
- Therapy
  - Enzyme replacement
  - Dosing by fat intake, body weight or symptoms
CF Related Diabetes (CFRD)

- Pancreatic endocrine insufficiency
- Symptoms - polyuria, polydipsia, weight loss, unexplained drop in lung function
- Screen yearly

CF- Bone and Joint Disease

- Osteopenia and osteoporosis in 40-60%
  - Vitamin D deficiency, calcium malabsorption, accelerated bone loss, hypogonadism, inactivity, low BMI, medicine effects, ? Direct CFTR effect
- Episodic arthritis
- Hypertrophic Pulm OsteoArthopathy

CF- GU effects

- Females
  - Endocervicitis, mucus distended cervical glands
  - Anovulatory
  - 20% infertile
- Males
  - Failure in transport, not production (CBAVD)
  - 98% infertile
  - MESA and ICSI
CF- Sweat glands

- Pronounced abnormality in Na, Cl homeostasis is well understood
  - No obstruction
  - No pathologic abnormalities
- Increased sensitivity to dehydration in hot weather
- Salty taste to sweat

Diagnosis

- Clinical evidence of CF
- Laboratory evidence of CF
  - Sweat test
  - Genetic test
  - Nasal potential

Diagnosis

- Sweat test (quantitative pilocarpine iontophoresis)
  - Minimum 50 mg sweat
  - Normal- sweat chloride <40 mmol/L, 40-60 borderline, > 60 abnormal
  - Needs to be done in an accredited lab
Diagnosis

- Genotyping
  - Commercial testing panels (25-97 most common mutations)
  - Sensitive and specific in Caucasians with typical disease
  - Less effective in the non-white population, non-classic phenotype
  - Full sequencing of the CFTR gene available (30 day turn-around time)
  - Becoming more important to know the mutation for therapy

Newborn Screening

- Illinois and most other states have started screening all newborns for cystic fibrosis.
- Combination of a blood test, sweat test and mutation panel.
- Improves nutritional status, growth and intellectual outcomes.
- Fewer life threatening complications and hospitalizations.
- Families avoid the average 6-15 month delay in diagnosis.


Therapy of CF

- Multidisciplinary team approach
- Airway clearance
- Antibiotics
- Bronchodilators
- Nutrition
  - Calorie maintenance
  - Enzyme replacement
  - Vitamin replacement
- Anti-inflammatory
  - Oral Steroids
  - Inhaled Steroids
  - NSAIDS
  - Azithromycin
- Mucolytics
- Lung Transplantation
Airway Clearance

Antibiotics

• Chronic oral suppressive
• Cycled, nebulized antibiotics
  – Inhaled preservative free tobramycin (TOBI)
  – Inhaled Aztreonam Lysine (Cayston)
  – Nebulized Colistin
• IV antibiotics for exacerbations

TOBI

• Randomized, double blind, trial of 520 patients, 300mg TOBI BID vs. placebo
• 28 days on therapy, 28 days off
• FEV₁ improved 10%
• Also improvements in pseudomonas density, exacerbation rate

Anti-inflammatory Therapy

- Oral steroids - delay progression of lung disease, BUT a high incidence of CFRD, growth suppression and cataracts
- High dose ibuprofen - slowed decline in lung function, most evident in 5-13 year olds with mild lung disease, have to monitor serum levels
- Inhaled steroids
  - 50% of patients are on them, difficult to show a benefit
- Azithromycin

Mucolytics

- rhDNase (Dornase alpha, Pulmozyme)
  - Extracellular DNA from wbcs can compose up to 10% of CF secretions and increase viscosity
  - In studies, decreased age adjusted risk of exacerbation, improved spirometry, QOL, dyspnea scores
- Hypertonic saline
  - ? mechanism of action (increased height of ASL, induction of cough, combination of both)
  - In studies, slight improvement in spirometry, significant decrease in exacerbations

Hypertonic Saline

The “Future” of Therapy for CF

- Therapy targeted at the specific defect caused by the mutation
- Potentiators
  - VX 770 (Ivacaftor)
- Correctors, Chaperones
  - VX 809 (Lumacaftor)
- Gene transfer
  - adenovirus or liposomes

Potentiators

Ivacaftor

Clinical trial of the potentiator VX 770 in CF patients with the G551D mutation

Correctors

Lumacaftor/Ivacaftor
Lumacaftor–Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR

Approved by FDA in July 2015


Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del

Gene Therapy

- **Pros**
  - Single gene defect
  - Only need 5-10% activity for normal CI transport function
  - Lung is accessible
  - Lungs are normal at birth

- **Cons**
  - Epithelium is difficult to transfect
  - Expression is short
  - Repeat administration not always possible
  - CFTR is multi-functional and not all of the functions are equally easy to correct.
  - Clinical success is difficult to measure

20+ trials to date, most are proof of concept

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Comparison of Therapies

<table>
<thead>
<tr>
<th>Improvement in FEV₁</th>
<th>Reduction in exacerbation rate</th>
<th>Cost/month</th>
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</thead>
<tbody>
<tr>
<td>Azithro</td>
<td>4.40%</td>
<td>40-50%</td>
</tr>
<tr>
<td>Flarvent</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.37%</td>
<td>?</td>
</tr>
<tr>
<td>Hypertonic Saline</td>
<td>3%</td>
<td>56%</td>
</tr>
<tr>
<td>rhDNase</td>
<td>5-16%</td>
<td>28-37%</td>
</tr>
<tr>
<td>TOBI</td>
<td>6-10%</td>
<td>26%</td>
</tr>
<tr>
<td>Cayston</td>
<td>6-10%</td>
<td>NS-40%</td>
</tr>
<tr>
<td>Ivacaftor</td>
<td>10%</td>
<td>55%</td>
</tr>
<tr>
<td>Orkambi</td>
<td>2.5-4%</td>
<td>30-40%</td>
</tr>
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Nutrition in CF

- Increased caloric demand
  - May need to take in 3500-4500 cal/day
- Decreased intake
- Malabsorption
- CF related diabetes
- Good nutritional status and weight >50% percentile associated with better outcomes

CF Foundation, Patient Registry, Annual Data Report 2015
A Nutritionist’s Advice to CF Patients

- Add calories by adding peanut butter or mayonnaise to foods
- Use gravies and sauces on vegetables, meats and starches
- Use salad dressing to marinate meats
- King sized candy bars are quick snacks for on-the-go
- Use heavy cream in place of milk
- Don’t push away the salt shaker
- Fast foods and prepared foods help to simplify meals. They not only pack in a lot of calories and fat, but tend to be high in sodium as well!

Adapted from: Beyond Lungs, Meeting the Needs of Adults With Cystic Fibrosis, ed A. McKenna, H. Goldswieg

Nutrition in CF

- Old school teaching is quantity over quality
- Oral supplements (Boost, Scandishakes—600 cal/8oz)
- Tube feeds
  - Place a feeding tube into the stomach
  - Run feeds (50% caloric requirement) overnight
  - Enzymes at the start of the feed
Lung Transplantation

- CF is the 3rd most common indication for transplant
- Slightly better outcomes
- Refer when FEV₁ <30% predicted

CF and the CF Foundation as a model for other diseases

- Genetics
- Fundraising
- Data collection
- Drug development

Conclusions

- Cystic fibrosis is an autosomal recessive disease caused by a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator gene.
- The details of how that mutation leads to the pulmonary disease are still unclear.
- Median survival is increasing into adulthood.
- Therapy includes airway clearance, antibiotics, mucolytics and nutritional support.
- Future therapy will focus on the processing and modulation of CFTR, restoring ion transport.
- Gene therapy is still years away.