Cystic Fibrosis Diagnosis and Treatment

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National Jewish Health
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Financial Disclosures

• Personal financial relationships with commercial interests relevant to medicine, within the past 3 years: NJH site PI for AstraZeneca. As faculty in institutions that are part of the CF Foundation TDN, I have been site PI for Nivalis, Rompex, and PTC Corp.

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Goals and Objectives

• Review the definitions of classic and non-classic Cystic Fibrosis (CF).
• Understand the genetic and non-genetic determinants of CF.
• Explore the diagnostic tests for CF.
• Update the understanding of disease pathogenesis and determinants of progression.
# Clinical Presentation of CF

## Pulmonary Symptoms
- Persistent cough
- Wheezing
- Shortness of breath
- Frequent pulmonary infections
- Purulent sputum

## Gastrointestinal Symptoms
- Pancreatic insufficiency
- Malabsorption
- Meconium ileus
- Obstructive cholestasis
- Pancreatidis
- Bowel obstruction
- Rectal prolapse

## Other Symptoms
- Salty sweat or skin
- Poor growth, weight loss
- Clubbing
- Nasal polyps, sinusitis
- Male infertility
Diagnosis by Clinical Triad

- Elevated Sweat Chloride
- Pancreatic Insufficiency
- Chronic Pulmonary Disease

Diagnosis by Mutation Analysis

- F508del
- Class 1-3 pancreatic insufficiency
- Class 4-5 pancreatic sufficiency
Diagnosis by Sweat Test

- Sweat Test
  - Pilocarpine iontophoresis
  - > 60 meq/L chloride
  - Inaccurate in first month of life
  - > 30 in newborn and young infant

- Other causes of elevated sweat chloride
  - Untreated hypothyroidism
  - Glycogen storage disease
  - Addison’s disease
  - Ectodermal dysplasia
Diagnosis by CFTR Genotyping

• Greater than 2000 different mutations in CFTR
  • Common mutations in USA: F508del, G542X
  • Mutations in China: I556V, M469V, E527N, F508del
  • Mutations in UAE and Middle Eastern Countries:
    • S4X (7%), S549N, 2043delG, 4016insG, S549R, I1234V*

• Conventional commercial genotyping
  • Genzyme: 86 mutations
  • Ambry: all coding mutations
  • Many cases are either one or two unknowns at this time
Newborn Screening for CF

• Mandated nationwide
• 66% of new diagnoses made in the first year of life
• Step-wise process:
  • Measurement of IRT
  • If elevated, then repeat IRT or CFTR genotyping
  • If still elevated or mutation detected, then sweat chloride test
• Sweat chloride test = best initial diagnostic test

<table>
<thead>
<tr>
<th></th>
<th>Age ≤ 6 mo</th>
<th>Age &gt; 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF is unlikely</td>
<td>&lt; 30 mmol/L</td>
<td>&lt; 40 mmol/L</td>
</tr>
<tr>
<td>Intermediate</td>
<td>30-59 mmol/L</td>
<td>40-59 mmol/L</td>
</tr>
<tr>
<td>Consistent with CF</td>
<td>≥ 60 mmol/L</td>
<td>≥ 60 mmol/L</td>
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Cystic Fibrosis Foundation, 2015.
Disorders affecting the airways with similar characteristics to CF or infections with Pseudomonas Aeruginosa

- Pan-bronchiolitis
- Chronic bronchitis
- Idiopathic bronchiectasis
- IgA, IgG, IgG subclass deficiencies
- COPD
- Patients with tracheostomy tubes
Diagnostic Complexity

• Patients not identified via NBS
  • Normal or equivocal sweat chloride test results
• 6.8% patients diagnosed ≥ 16 years old
• Clinical presentation
  • Often diagnosed in adolescence or adulthood
  • Genetic mutation – less common defect
  • Mild disease – respiratory, GI, pancreatic
  • Atypical symptoms – infertility, sinusitis
  • Infection with typical CF respiratory pathogens

Atypical CF Diagnosis

- Often occurs in adolescence
- PMH in retrospect of CF-like symptoms
- Lung, sinus, liver, male infertility

**Diagnosis by Mutation Analysis**

- One identifiable mutation plus intron modifier
- Frequently Class 4-5 pancreatic sufficiency
What Has Stayed the Same Since 1996?

- Sweat testing is still the gold standard test to confirm a CF diagnosis
- The CF phenotype remains pretty much the same
- There are still cases with atypical clinical features and indeterminate laboratory test results that remain hard to classify
- Nasal PD testing has become standardized but is still used infrequently to confirm or rule out a CF diagnosis
F508del is the most common mutation
How much is enough?

• Depends....
  • CFTR Cl- channel activity
  • CFTR and Na+ transport
    • CFTR bicarbonate/GSH/other ion transport
  • CF mucociliary transport
  • CF inflammation
  • CF mucus abnormalities
  • CF bacterial susceptibility
Lessons from nature in vivo

• 5T allele reduces CFTR expression
  • 6-11%
• CBAVD and male infertility
  • 5-10%
• Partial function CFTR mutations
  • 4.8%
Estimates in vitro

• Chloride transport after gene transfer
  • 6-10%

• Normalization of sodium transport after gene transfer
  • 100%

• Correction of inflammation
  • Xx%
Nasal Potential Difference

• Measures the electrical potential across cells and cell membranes
• Nasal measurements are surrogates for lung airways
• Baseline PD is negative due to sodium absorption through ENaC
• PD depolarizes with sodium blockade and hyperpolarizes with chloride secretion
Excessive sodium absorption and Pancreatic function

Knowles, et al.  
*Hum. Gene Ther.* 1995
Chloride secretion and pancreatic function

Knowles, et al.

Hum. Gene Ther. 1995
Sweat Chloride in Newborns

Sweat [Cl⁻] Mmol/L

Percent CFTR activity

* Farrell, 1996
** Denver, 2005

(F. Accurso; CF-TDN)
Can we repair the mutant CFTR with drugs alone?

• Readthrough premature stop codons
• Correct protein trafficking
• Potentiate or stimulate chloride conductance
• Increase synthesis
EXAMPLE OF A NON-CF NPD

- Amiloride (to block sodium)
- Low chloride (to detect open channels)
- Isoproterenol (to activate CFTR)

- Basal NPD
- Ample chloride transport

Time (min)

Property of Presenter
Not for Reproduction
EXAMPLE OF A CF NPD

- Basal NPD
- Absent chloride response

- Amiloride
- Chloride-free
- Isoproterenol
Induction of nasal epithelial chloride transport by phenylbutyrate in patient who is homozygous for ΔF508 CF
What Has Changed Since 1996?

- The number of identified CFTR mutations has increased from 500 in 1996 to 2000+ in 2017
- The number of mutations classified as CF-causing has increased from 24 in 1996 to 82 in 2015
- Sequencing of the CFTR gene is now widely available and widely used
- Prenatal and pre-conceptual carrier testing recommended by ACOG for routine use in 2001 and now being used with increased frequency.
What Has Changed?

Newborn screening has gone from 3 states (Colorado, Wisconsin and Wyoming) in 1996 to all 50 states and the District of Columbia in 2011

• The number of cases in which the diagnosis is suggested by a NBS test result has gone from 5.7% in 1998 to 42.7% in 2008 to 83% in 2013

• Expanded understanding of genotype-phenotype correlations

• Recognition of the *CFTR*-related metabolic syndrome (CRMS) *or* CFSPID: In 2013, 8.4% of patients entered in the CF registry had a diagnosis of CRMS

• Resetting of intermediate sweat chloride values in infancy
Survival Continues to Improve – BUT . . .

CFF Patient Registry, 2015


... We Have More To Do To Advance Quality of Care

**Intervene Earlier**
- Target CF lung disease before symptoms occur
- Target genetics to optimize pulmonary and nutritional status
- Prevent bronchiectasis and loss of lung function
- Promote good nutrition

**Develop Comprehensive Treatment Plan (CTP) from Early Age**
- Address comorbidities
- Add exercise to CTP
- Address adherence
- Plan transition to adult care
Need to Identify CF and Intervene Early

• Newborn Screening (NBS)
  • Sweat Cl⁻ test
  • Implemented nationwide 2010
  • Add CFTR genetic analysis
    • If sweat chloride is intermediate
  • Add CFTR physiologic testing
    • If genotype undefined or MVCC

• Late Diagnosis
  • Normal/equivocal sweat Cl⁻
  • 6.8% diagnosed ≥ 16 yo
  • Clinical presentation
    • Mild disease
    • Atypical symptoms

* mmol/L. MVCC, mutation of varying clinical consequence

Early Diagnosis by Newborn Screening May Improve Survival

- 27,703 patients reported to 1986-2000 CFF Registry
- 4 diagnostic groups
  - Prenatal or neonatal screening (SCREEN)
  - Meconium ileus (MI)
  - Positive family history (FH)
  - Symptoms other than meconium ileus (SYM)
- Compared to SCREEN, those in MI and SYM groups had increased risk of
  - Shortened survival
  - Pseudomonas aeruginosa
  - $\text{FEV}_1 < 70$

Early Growth Predicts Childhood Survival

- Prospective, observational study using CFF Registry data
  - Tracked weight-for-age percentile (WAP) and outcomes

Results
- WAP at age 4 positively associated with height-for-age throughout childhood
- If WAP at age 4 > 10%, better lung function at ages 6-18
- If WAP at age 4 > 50%, fewer acute pulmonary exacerbations at age 18
  - Also, fewer total days in hospital
  - Lower rates of impaired glucose tolerance or diabetes

Infants Do Not Catch Up to Length Percentile at 1 Year

- FIRST study growth was normal at birth
- Growth (weight, length) declined at 2 months
- Some catch-up growth by 12 months
  - Weight increased
  - Length did not fully recover

<table>
<thead>
<tr>
<th></th>
<th>Weight WHO Percentile</th>
<th>Length WHO Percentile</th>
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<tbody>
<tr>
<td>At birth</td>
<td>41</td>
<td>61</td>
</tr>
<tr>
<td>At 2 months</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>At 12 months</td>
<td>58</td>
<td>39</td>
</tr>
</tbody>
</table>
BONUS Study Also Found Risk for Growth

197 infants (2012-2014)

At 3 months:
- 35% at risk for weight
- 34% at risk for length

At 12 months:
- 9% < 10th percentile for weight
- 27% < 10th percentile for length

Conclusion:
- Despite improvements in weight gain in the 1st year of life, stunting persists in the majority

BONUS = Baby Observational and Nutrition Study
Leung DH, et al. Abstr #575 NACFC
Risk Factors for Mortality < Age 18 in CF

- 3,880 patients
  - Enrolled in ESCF with at least 1 visit annually at ages 3, 4, & 5 years
  - Also linked to CFFPR data
  - Born 1991-1995
- 191 (5.7%) died < 18 years of age
  - Median age at death, 13.4 +/- 3.1 years

**Significant Risk Factors for death (regardless of FEV₁ at age 6-8 years)**

<table>
<thead>
<tr>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clubbing</td>
</tr>
<tr>
<td>Crackles</td>
</tr>
<tr>
<td>Female sex</td>
</tr>
<tr>
<td>Unknown CFTR genotype</td>
</tr>
<tr>
<td>Minority race or ethnicity</td>
</tr>
<tr>
<td>Medicaid insurance (proxy of low socioeconomic status)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> on ≥ 2 cultures</td>
</tr>
<tr>
<td>Weight-for-age &lt; 50th percentile</td>
</tr>
</tbody>
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It Is Critical to Develop a Comprehensive Treatment Plan from Early Age: 6 Key Components

- Treat CF lung disease
- Target genetics to optimize pulmonary and nutritional status
- Add exercise!
- Address adherence
- Address comorbidities:
  - Anxiety and depression
  - Diabetes
  - GI problems
- Plan transition to adult care
CFTR Protein Is Key to Proper Lung Function

• Cystic fibrosis transmembrane conductance regulator (CFTR) protein:
  • Forms the major transport channel for Cl⁻ and HCO₃⁻
  • Determines mucus viscosity
• CFTR proteins must be present and functioning well for good lung function
• Over 1,800 mutations in the CFTR gene
  • Mutated CFTR protein → lower or absent for Cl⁻ transport

Systems Affected by Mutations in CFTR Protein

- Lungs
- GI Tract
- Pancreas
- Liver

Abnormal CFTR Leads to Repeated Infections and Inflammation

Abnormal CFTR
- Reduced airway surface liquid
- Impaired mucociliary clearance

Routine clearance of pathogens from the lungs is compromised

Patients at risk of repeat pulmonary infections and prone to exaggerated inflammatory response
Downward Spiral of Lung Function from Repeated Infections and Inflammation

Abnormal CFTR
- Reduced ASL
- Impaired mucociliary clearance

Inflammation → Infection → Obstruction → Structural damage

Inflammation → Infection → Obstruction → Bronchiectasis

Inflammation → Infection → Obstruction → Pulmonary insufficiency

Inflammation → Infection → Obstruction → Respiratory failure
Changes in Prevalence of Respiratory Pathogens


- **S. aureus**
- **P. aeruginosa**
- **MRSA**
- **H. influenzae**
- **S. maltophilia**
- **MDR-PA**
- **Achromobacter**
- **B. cepacia complex**

CFF Patient Registry, 2015
Respiratory Pathogens – Difference by Age

Prevalence of Respiratory Microorganism, by Age Cohort, 2015

CFF Patient Registry, 2015
Airway Microbiome USA CFTDN study 2017

• Characterization of microbiota in CF bronchoalveolar lavage fluid (BALF)
• 146 CF (13 centers) and 45 disease controls
• If CF < 2yo, *Streptococcus* predominates; if ≥ 6 yo, traditional CF pathogens
• Sequencing identified a dominant taxon in 24% of culture-negative BALF (*Streptococcus* or *Prevatella*)
• Microbial diversity and relative abundance of *Streptococcus*, *Prevotella* and *Veillonella* were inversely associated with airway inflammation
• Microbial communities were distinct between CF and non CF BALF

Targeting the Genetic Defect

- Gene therapy
- Gene editing
- RNA repair
- CFTR modulation
  - mRNA modulation
  - Correction of mRNA translation to protein
  - Potentiator
  - Inhibitor of proteostasis
  - Corrector
  - Stop codon read-through drug

# CF Mutation Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Impact on CFTR</th>
<th>Mutation Example</th>
<th>Potential Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No functional CFTR created</td>
<td>G542X, W1282X</td>
<td>Gene therapy RNA correction Read-through drug</td>
</tr>
<tr>
<td>II</td>
<td>Processing defect</td>
<td>Phe508del</td>
<td>Corrector + Potentiator</td>
</tr>
<tr>
<td>III</td>
<td>Regulation defect</td>
<td>G551D</td>
<td>Potentiator</td>
</tr>
<tr>
<td>IV</td>
<td>Decreased conductance</td>
<td>R117H</td>
<td>Potentiator</td>
</tr>
<tr>
<td>V</td>
<td>Reduced synthesis of CFTR</td>
<td>3849+10kbC→T A455E</td>
<td>Corrector Potentiator</td>
</tr>
<tr>
<td>VI</td>
<td>Altered channel stability</td>
<td>4326delTC</td>
<td>Potentiator Proteostasis inhibitor</td>
</tr>
</tbody>
</table>

## CFTR Modulators

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type</th>
<th>Mechanism of Action</th>
<th>Phase III</th>
<th>Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivacaftor</td>
<td>Potentiator</td>
<td>Increases channel opening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ataluren</td>
<td>Read-through</td>
<td>Enables the formation of a functioning protein</td>
<td>Failed Phase III; research program ended</td>
<td></td>
</tr>
<tr>
<td>Lumacaftor</td>
<td>Corrector</td>
<td>Moves defective CFTR protein to proper place in cell membrane and</td>
<td></td>
<td>Lumacaftor + Ivacaftor</td>
</tr>
<tr>
<td>VX-661 (Tezacaftor)</td>
<td>Corrector</td>
<td>Improves its function as a chloride channel</td>
<td></td>
<td>VX-661 + Ivacaftor*</td>
</tr>
</tbody>
</table>

*Positive results from two Phase III trials were reported March 28, 2017
NDA to be submitted to FDA in 3rd quarter 2017