Atypical Cystic Fibrosis

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Conflict of interest

• None
Outline

• To discuss the diagnosis of atypical cystic fibrosis in screened and unscreened patients
• To discuss the ramifications of CFTR variant mutations
• To discuss what it means to be diagnosed with CFTR-related metabolic syndrome (CRMS)
• To discuss how to recognize atypical CF and CFTR related-disorders
Cystic Fibrosis Milestones

- Distinct Clinical Entity (1938)
- Sweat Gland Defect (1953)
- Pilocarpine Iontophoresis Sweat Test (1959)
- Phenotypic Heterogeneity (1960 – 1989)
- CFTR-Related Disorders (1990 – 2004)
Although a single gene defect-

CF can have a wide spectrum of symptoms and outcomes even among people with the same mutation.
How is the diagnosis of cystic fibrosis usually defined?

• Identification of 2 disease causing CFTR mutations and laboratory evidence of CFTR dysfunction
  • Sweat chloride- gold standard
  • Alternative ways of measuring CFTR function
    • Nasal epithelial potential difference (NPD)
    • Intestinal current measurement (ICM)

• Demonstration of the CF phenotype in at least one organ system
Problems with the CF Diagnosis

• Patients with a “typical” CF phenotype may have no identified mutations
• Individuals with two mutations may be asymptomatic
• Individuals identified by newborn screening may be asymptomatic or mildly symptomatic for long periods of time
• The relationship between CF and CFTR related disorders (ABPA, bronchiectasis, pancreatitis) creates diagnostic dilemmas
What to do with CFTR mutations of unknown significance-
Does the patient have CF?
CFTR2 Project

• CFTR2 is a website that provides information for patients, researchers, and the general public about specific variants in the CFTR gene.

• For each variant or variant combination included in the database, the website will provide information about:

  1. Whether the variant or variant combination is CF-causing, and
  2. Information about sweat chloride, lung function, pancreatic status, and Pseudomonas infection rate in patients in the CFTR2 database with this variant or variant combination.
CFTR2 project: evaluates CFTR mutations of unknown significance

**Summary:** G542X is seen in 3474 patients in our worldwide CF database. Based on the combination of clinical and functional evaluation, this is a mutation that would cause CF. Based on the patients we have reviewed we would expect this mutation would be associated with pancreatic insufficient CF.

**G542X mutation**

- **CF-causing mutation**
- **Mutation of varying clinical consequence**
- **Non CF-causing mutation**

**Summary:** D1152H is seen in 555 patients in our worldwide CF database. The clinical and functional analysis of this mutation shows that this mutation has variable expression or penetrance.

**D1152H mutation**

- **CF-causing mutation**
- **Mutation of varying clinical consequence**
- **Non CF-causing mutation**

**Summary:** R31C is a mutation that has been evaluated and does not cause CF. This determination is based on evaluation of clinical characteristics of patients carrying this mutation, functional testing of this mutation, and finding this mutation (combined with a CF-causing mutation) in individuals who do not have CF.

The determination of non CF-causing does not exclude the possibility that this mutation may contribute to CF-like symptoms in certain individuals. In some cases, patients with this mutation (combined with a CF-causing mutation) may develop mild symptoms in select organ systems and/or be diagnosed as having a CFTR-related disorder (CFTR-RD; see FAQs). However, this mutation is not expected to result in symptoms that fulfill the diagnostic criteria for CF.

**R31C mutation**

- **CF-causing mutation**
- **Mutation of varying clinical consequence**
- **Non CF-causing mutation**
Other dilemmas complicating the CF diagnosis
The diagnosis of CF must include evidence of CFTR dysfunction.

• What should be done if the sweat chloride test is intermediate or normal in a patient with a clinical CF phenotype or CF mutations of unknown significance

• Alternative ways of measuring CFTR function
  • Nasal epithelial potential difference (NPD)
  • Intestinal current measurement (ICM)
Nasal epithelial potential difference as a test for CFTR function

**Normal response**

- Normal response

**Cystic Fibrosis response**

- Cystic Fibrosis response
Intestinal current measurement (ICM) as a test for CFTR function

- ICM is an important tool for functional assessment in CFTR mutations of unknown clinical relevance – used in Europe
- Transepithelial ICM is measured ex vivo in superficial rectal biopsy material
- No sedation or bowel prep, even in newborns
- forskolin/IBMX + carbachol + histamine used to stimulate chloride transport in rectal tissue in Mini-Ussing chambers
- International SOPs available
- In limited studies, high degree of sensitivity and specificity
Sweat chloride levels correlate with intestinal current measurements.
How to diagnose CF with neonatal screening
On the basis of a preponderance of evidence, the health benefits to children with CF outweigh the risk of harm and justify screening for CF.
Currently at least 64% of new CF diagnosis in the US occur in asymptomatic or minimally symptomatic infants following a positive newborn screening result.

### Age at Diagnosis for all People with CF in the Registry, 2013

- Under 1 year, 65.7%
- 1 year old, 6.6%
- 2-15 years old, 20.8%
- 16 years and older, 6.8%
- Prenatal, 2.3%
- Under 1 month, 29%
- 1 to 3 months, 13.2%
- 4 to 6 months, 12.2%
- 7 to 11 months, 9.0%
Serum immunoreactive trypsinogen used in newborn screening

- Elevated in infants with CF and in CF carrier - pancreatic ducts are partially blocked leading to abnormal enzyme drainage
Newborn screening strategies-

- IRT+/IRT+ --------------------------sweat test
- IRT+/DNA+ --------------------------sweat test
- IRT+/IRT+/DNA+ ------------------sweat test
- IRT+/DNA-/IRT+ -------------------sweat test
- IRT+/DNA-/VHIRT+ ---------------sweat test

- DNA analysis usually involves a panel of 23-40 of the most common CF-causing mutations.

- IRT- immunoreactive trypsinogen

Farrell et. al., J. Ped. 2017
Confirmation of a CF diagnosis after a positive newborn screen result- consensus guidelines

• Sweat chloride $>$ 60 mmol or 2 CF-causing mutations plus sweat CL $>$ 30 mmol

• Variable or uncharacterized mutations, sweat CL $>$ 30 mmol and a positive nasal potential difference or intestinal current measurement

Farrell et. al., J. Ped. 2017
Things to remember when diagnosing CF by NBS

• Identification of 2 CF-causing mutations is consistent with a CF diagnosis in infants with a positive newborn screen, CF symptoms or positive family history, however a positive sweat test (x1) is still needed to confirm the diagnosis.
• Absence of 2 CF-causing mutations does not exclude a CF diagnosis.
• A genetic analysis included as part of NBS must not be relied upon for a conclusive diagnosis and/or genotyping.

Farrell et. al., J. Ped. 2017
The dilemma of CFTR-related metabolic syndrome (CRMS) and CF screen positive, inconclusive diagnosis (CFSPID) with newborn screening
Clinical Presentation of CF- algorithm

Positive newborn screen
Signs and/or symptoms of CF
Family history

Sweat Chloride test

>-60mmol/L
>30-59mmol/L
<29mmol/L

CFTR genetic analysis

2 CF causing mutations
CFTR genotype undefined or of known varying clinical consequence
No CFTR mutation

CFTR physiological testing
NPD or ICM

CFTR dysfunction
Testing unavailable or unequivocal
CF function preserved

CF diagnosis

CF diagnosis not resolved

CF unlikely

Farrell et. al., J. Ped 2017
CFTR-related metabolic syndrome (CRMS) and CF screen positive, inconclusive diagnosis (CFSPID)

- Infant with a positive newborn screen for CF and either sweat chloride <30mmol/L and 2 CFTR mutations, at least 1 with unclear phenotypic consequences
- Infant with intermediate sweat chloride value (30-59mmol/L) and 1 or 0 CF causing mutations

CRMS/CFSPIDS

- Individuals with CRMS/CFSPID may never develop CF symptoms but should be referred to CF center to establish a follow-up plan.
- Usually they are pancreatic sufficient.
- 11% may have at least 1 positive pseudomonas culture during first year of life.
- 4-11% will convert to a CF diagnosis over 3-5 year follow-up.
Prospective study of infants with CFSPID

82 infants met criteria for a CFSPID diagnosis

3 year follow-up:

• 9 infants (11%) converted to a CF diagnosis

• CF diagnosis based on:
  
  4 genotype alone
  
  3 genotype + sweat test
  
  2 sweat test alone

• Mean age at positive sweat test was 21 months

Ooi et al: Pediatrics June 2015
Management of infants with CRMS/CFSPIDS

• Initial assessment by a CF specialist by age 2 months
• Comprehensive history and physical
• Extended CFTR gene analysis
• Stool elastase
• Oropharyngeal culture (if positive for P. aeruginosa, may treat per protocol)
• Repeat sweat test at 6 month
• Induced sputum or BAL (rarely indicated)
California neonatal CF screening experience

In California- 2,573,293 newborns screened from 2007-2012

• Three step model was used:
  • Immunoreactive trypsinogen was measured from a newborn dried blood spot
  • Samples with IRT ≥62 ng/mL (top 1.6%) were screened for 28-40 selected CFTR mutations
  • DNA sequencing was performed on specimens found to have only 1 mutation in step 2.
  • Infants with ≥2 mutations/variants were referred to CF care centers for diagnostic evaluation and sweat chloride testing.
  • Infants with one mutation were considered carriers
California Results

- 40,646 infants - positive IRT on NBS
- These newborns were screened for 28-40 selected CFTR mutations
  - 345 children with CF picked up by NBS
  - 28 children with CF were missed by NBS
  - 533 children were categorized as CRMS
  - 1617 children were picked up as carriers of a mutation

Kharrazi et al., Pediatrics, 2015
Presentation of atypical CF in individuals not screened in the neonatal period
The diagnosis of Atypical CF in people not screened by newborn testing can be difficult to make

- Sweat chloride tests may be inconclusive
- The pathogenicity of the CFTR mutation may be uncertain
- There can be differential expression of CFTR protein or modifier effects
- Pancreatic sufficient with some CF clinical phenotype
Features of Atypical CF:

- Normal or borderline sweat chloride (40-60 mEq/L)
- Pancreatic sufficient with mild lung disease
- A CF phenotype in at least one organ system, including:
  - Focal biliary cirrhosis and portal hypertension
  - Recurrent pancreatitis
  - Chronic rhino-sinusitis
  - Nasal polyposis
  - Infertility due to congenital bilateral absence of the vas deferens (CBAVD)
For example, Class IV and V mutations account for 10% of CF mutations. These individuals can have partial CFTR function, pancreatic sufficiency, and mild lung symptoms.

<table>
<thead>
<tr>
<th>Class</th>
<th>No synthesis</th>
<th>Block in processing</th>
<th>Block in gating</th>
<th>Altered conductance</th>
<th>Reduced synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>G542X</td>
<td>F508del</td>
<td>G551D</td>
<td>D1152H</td>
<td>3849+10kbC→T</td>
</tr>
<tr>
<td>I</td>
<td>12%</td>
<td>87%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

% of patients with an allele in this class

Schematic courtesy of Dr. Michael Boyle
Figure 3. Sweat chloride levels versus predicted CFTR activity. Data are extracted from References 10, 79, and 82. CFTR activity is predicted by genotype–phenotype relationships and in vitro studies. Normal individuals are assumed to have 100% CFTR activity. Carriers are assumed to have 50% CFTR activity. Sweat chloride levels are more elevated in patients with two mutations that are usually associated with pancreatic insufficiency (PI) compared with patients who carry two mutations, one of which has been associated with pancreatic sufficiency (PS). Patients with two mutations and congenital absence of the vas deferens (CBAVD) as their sole clinical manifestation of CFTR mutations have sweat chloride levels intermediate between those of patients with PS and carriers. Data are expressed as mean values ± 1 SD.

Published in: Steven M. Rowe; Frank Accurso; John P. Clancy; Proc Am Thorac Soc 2007, 4, 387-398.
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Idiopathic recurrent pancreatitis- can be a CF variant

• 10-20% of people with this condition have 2 CFTR mutations, one of which is usually a mild mutation.
• They may have no lung disease
• Normal or borderline sweat chloride levels
• Abnormal nasal potential differences
Nasal polyposis and chronic rhinosinusitis - a CF variant

Nasal polyposis is unusual in children - CF testing should be done in children found to have nasal polyposis
Nasal polyposis and chronic rhinosinusitis-

- Sino-nasal manifestations are prevalent in almost all patients with CF, whether represented by signs, symptoms, or radiologic findings
- **Nasal polyps** are the most frequently encountered findings on physical examination and may occur in up to 86% of patients
- Virtually all CF patients demonstrate radiological evidence of sinus disease, but the extent of disease does not correlate with symptom severity
Congenital bilateral absence of the vas deferens (CBAVD)

• 1-2% of infertile males have CBAVD
  • Present with azoospermia
• 50% of males with CBAVD have CFTR mutations
• Often caused by Class IV and V missense and splice variants
• Can be caused by variation in alleles in the poly-T tract sequence of intron 8 of the CFTR gene
  • R117H/7T can result in congenital bilateral absence of the vas deferens
  • Also can be associated with chronic sinusitis and
  • abnormal nasal epithelial potential differences

http://www.nchpeg.org/nutrition/index
Figure 3. Sweat chloride levels versus predicted CFTR activity. Data are extracted from References 10, 79, and 82. CFTR activity is predicted by genotype–phenotype relationships and in vitro studies. Normal individuals are assumed to have 100% CFTR activity. Carriers are assumed to have 50% CFTR activity. Sweat chloride levels are more elevated in patients with two mutations that are usually associated with pancreatic insufficiency (PI) compared with patients who carry two mutations, one of which has been associated with pancreatic sufficiency (PS). Patients with two mutations and congenital absence of the vas deferens (CBAVD) as their sole clinical manifestation of CFTR mutations have sweat chloride levels intermediate between those of patients with PS and carriers. Data are expressed as mean values ± 1 SD.

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Idiopathic bronchiectasis in older people - a CF variant

- Mild pulmonary disease and idiopathic bronchiectasis in older patients
- CF has been diagnosed in individuals as old as 70 yrs
- Pulmonary disease usually less severe but chronic Pseudomonas aeruginosa infection can be found
- Can be misdiagnosed as asthma or chronic obstructive pulmonary
Who gets the diagnosis of CFTR-related disorder?

- Individuals with some but not all CF diagnostic criteria who were not screened in the neonatal period
- Individuals who had a negative newborn screen but developed some but not all of the CF diagnostic criteria
- Will have a mono-symptomatic clinical entity with CFTR dysfunction
  - Congenital absence of the vas deferens
  - Pancreatitis
  - Bronchiectasis

Farrell et. al., J. Ped, 2017
Homozygote F508del mutations are associated with high sweat chlorides and little or no CFTR protein function.

Class IV and V mutations associated with more CFTR Function and patient are usually pancreatic sufficient.
Non-CFTR factors that can affect disease severity in CF

• Exposure to secondhand smoke
• Immune response to bacterial infections may vary among people with the same CF mutation
• Genetic modifiers other than CFTR that may worsen or decrease disease severity
The role of CFTR in other diseases: COPD

Cigarette smoke induces systemic defects in cystic fibrosis transmembrane conductance regulator function

Raju, et al., 2013
Summary

- Adults diagnosed with atypical CF usually have longer life expectancies than individuals with classic CF.
- However, the long-term outcome for many individuals with atypical CF is unknown.
  - It is important to counsel patients about the possibility of future illness.
- There are wide variations in phenotypes of CF among individuals and family members, even with the same genotype.
- Environmental factors such as secondhand smoke exposure can negatively impact symptoms.
Summary

• The CFTR spectrum of dysfunction is much larger than previously thought
• Most patients on the mild or atypical end of the spectrum will have 1+ mutations with residual CFTR function
• CFTR molecular testing is complicated!
  • Genetic counselors can help sort out which test to order and how to interpret the results
  • Carefully reading the lab report front-to-back is crucial for interpretation
• There are no hard-and-fast rules about which mutations will result in mild/atypical phenotypes…
  • …but certain mutations are seen more frequently in these populations than others
  • R117H, 5T, “polymorphisms,” and variants of unknown significance
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