Cystic Fibrosis and CF Newborn Screening in Texas

Grand Rounds
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John Saito, MD, FAAP, FCCP
Cook Children’s Physician Network
CF Newborn Screening Director

Cystic Fibrosis
• Genetic disease
• Altered gene on long arm of chromosome 7
• Autosomal recessive inheritance
• Incidence by ethnic background
  - Caucasian ~1/3000
  - Hispanic ~1/6000
  - African American 1/10,000
• ~30,000 people in the USA
• ~70,000 people worldwide

Source: 1 Cystic Fibrosis Foundation, 2008

Cystic Fibrosis Transmembrane Conductance Regulator
(Chloride Ion Channel)

• Results in defective production of CFTR protein
  • Faulty transport of salt in multiple organ systems

Wild-type: CFTR is transcribed into mRNA followed by posttranslational modifications including proper folding, glycosylation, and trafficking via the Golgi apparatus to cell membrane where it functions as a regulated chloride channel.

Class 1 mutations (G542X), contain premature stop mutations that create truncated mRNA.

Class 2 mutations (F508), misfolded and unable to escape the endoplasmic reticulum.

Class 3 mutations (G551D), reach the cell membrane but the channel is not properly activated.

Class 4 mutations (R347P), reach the cell surface, channel can be activated but have decreased chloride conductance.

Class 5 mutations (3849 10 kb C → T), incorrect splicing resulting in decrease abundance of CFTR (milder phenotype).

Source: Cystic Fibrosis Foundation Search for a Cure, 2004

Pulmonary Impact of CFTR Defect

Video: Impaired Mucociliary Clearance

Bronchoscopy of CF airway with thick mucus

Source: Cystic Fibrosis Foundation Search for a Cure, 2004
Pulmonary Disease in CF

- Normal at birth
- Impaired mucociliary clearance
- Bronchial obstruction
- Infection
- Inflammation
- Bronchiectasis

Pulmonary Disease and Lung Function Decline

**Median Percent Predicted FEV1 vs Age, Knudson Equations 1990**

- FEV1 percentage predicted has improved 10 percentage points for CF patients ages 6 to 36 years since 1990.

**Median Percent Predicted vs. Age, 1990 and 2007**

- FEV1 percentage predicted has improved 10 percentage points for CF patients ages 6 to 36 years since 1990.

CF Mortality

- Infants and children are still dying of CF

Intestinal Malabsorption in CF

- Multi-Factorial
  - Abnormal CFTR function in pancreatic duct
  - Deficiency of pancreatic enzymes
  - Deficiency of pancreatic bicarbonate
  - Increased falciparum of bile salts
  - Secretion of bile and enterohepatic bile salts
  - Uptake and transport of long-chain fatty acids
  - Altered mobility and increased small bowel transit time
  - Structural abnormalities from previous surgery

- 85% CF pts have exocrine pancreatic insufficiency with malabsorption of fat and fat-soluble vitamins (A, D, E, K)

- Pancreatic Enzyme Replacement Therapy (PERT)
  - Enzyme doses up to 2,500 IU lipase/units/day
  - Patient should tolerate a normal to high-fat diet without abdominal pain, distension, or abnormal or excessively frequent fatty stools.
74% are diagnosed by 2 years of age.

**Importance of Early Diagnosis**
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."

Newborn Screening...

Is an essential, preventive public health program for early identification of disorders that can lead to catastrophic health problems.

The cost of these disorders, if left untreated, is enormous, both in human suffering and in financial terms.
**Brief Review of the Texas Newborn Screening Program**

- 1963 – Phenylketonuria (PKU) pilot
- 1965 – Mandated PKU screening
- 1978 – Added Galactosemia & Homocystinuria screening
- 1980 – Added Congenital Hypothyroidism screening, Recommended second screen
- 1983 – Discontinued Homocystinuria screening, added Hemoglobinopathy screening, Required second screen
- 1989 – Added Congenital Adrenal Hyperplasia screening
- 1995 – Added second-tier DNA testing for hemoglobinopathies
- 2002 - Expanded NBS Task Force recommended the program expand with MS/MS technology (add 4 disorders)
- 2003 – Legislation to expand program did not pass

**Review: Texas NBS Program**

- 2008: Received ~796,000 specimens
  - Specimens Assayed and Reported: ~791,000
  - ~ 4,800 unsatisfactory specimens (~0.60%)
  - Avg: 2,527 specimens/day
- Two screening tests for each baby born in Texas
  - 24 – 48 hours of age
  - 1 – 2 weeks of age
- Infants testing positive receive prompt confirmatory testing.

**Cystic Fibrosis: Overarching Assumptions**

- IRT/IRT/DNA model
- DSHS workload estimates:
  - FY10: 818,000 screens
  - FY11: 828,000 screens
- Estimate 82-94 diagnosed cases annually

**Testing Algorithm (IRT/IRT/DNA)**

- Measure IRT levels on both 1st and 2nd screens
- Elevated IRT levels on both screens triggers a DNA test
- Fail safe protocol:
  - If 2nd screen not received within 30 days after birth, reflex to DNA

**Cystic Fibrosis Newborn Screening Algorithm**

1. 1st Screen Blood Spot
2. 2nd Screen Blood Spot
   - Elevated IRT
   - Normal IRT
   - Normal CF Screen
3. CFTR Mutational Analysis
   - Elevated IRT & 2 CFTR mutations
   - Elevated IRT & 1 CFTR mutation
   - Elevated IRT 0 CFTR mutation

DSHS Positive CF NBS
Cystic Fibrosis Newborn Screening Algorithm

1st Screen Blood Spot

- Normal IRT
- Elevated IRT
- "Indeterminate"

Causes for elevated IRT:
- Many unaffected infants have an elevated immunoreactive trypsinogen (IRT) level on the first specimen.
- The second screening specimen (collected after 7 days of age) is required to determine if result is significant.
- Please repeat the newborn screen.

2nd Screen Blood Spot

- Normal IRT
- Elevated IRT

Causes for elevated IRT:
- Perinatal asphyxia
- Septicemia
- Trisomies (13, 18 & 21)
- Obstructive liver disease
- Biliary atresia
- Necrotizing enterocolitis (NEC)
- Intestinal perforation
- Hydronephrosis

Cystic Fibrosis Newborn Screening Algorithm

DSHS Positive CF NBS

Elevated IRT

2 CFTR mutations

Elevated IRT

1 CFTR mutation

Elevated IRT

0 CFTR mutation

Inconclusive

No further evaluation necessary unless clinically indicated.

Although there is a minimal risk for Cystic Fibrosis (CF) in the absence of detected mutations, an elevated immunoreactive trypsinogen (IRT) result may be indicative of CF due to a mutation not included in the 40-mutation panel. Recommend sweat testing and possible genetic evaluation only if clinically indicated.

CFTR Mutation Panel

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Normal IRT</th>
<th>Elevated IRT</th>
<th>Inconclusive</th>
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<tbody>
<tr>
<td>AF508</td>
<td>R334W</td>
<td>Q493X</td>
<td></td>
</tr>
<tr>
<td>a507</td>
<td>R347P</td>
<td>3905insT</td>
<td></td>
</tr>
<tr>
<td>G542X</td>
<td>711+1G-T</td>
<td>V520F</td>
<td></td>
</tr>
<tr>
<td>G555G</td>
<td>1899+1G-A</td>
<td>5490 (T&gt;G)</td>
<td></td>
</tr>
<tr>
<td>W1282X</td>
<td>2186delA</td>
<td>3460delT</td>
<td></td>
</tr>
<tr>
<td>N1353X</td>
<td>1076delT</td>
<td>E60X</td>
<td></td>
</tr>
<tr>
<td>R555X</td>
<td>3849+10kbC</td>
<td>7849+4A-V</td>
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</tr>
<tr>
<td>G621+1G-T</td>
<td>2789+5G-D</td>
<td>R347H</td>
<td></td>
</tr>
<tr>
<td>R117H</td>
<td>3696delIC</td>
<td>5490 (A&gt;C)</td>
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</tr>
<tr>
<td>T171X-50G</td>
<td>1102X-50G</td>
<td>5490 (A&gt;C)</td>
<td></td>
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<tr>
<td>A455E</td>
<td>S549N</td>
<td>Y1092X C-G</td>
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<tr>
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<td>3675delA</td>
<td>Y122X</td>
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<td>R1162X</td>
<td>2183delG</td>
<td>P508C</td>
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<tr>
<td>G555E</td>
<td>3115delN</td>
<td>Y1383delT/171T</td>
<td></td>
</tr>
</tbody>
</table>
**Abnormal Screen Report**

**Positive CF NBS: What Now or Next?**

DSHS case will be created for case management if:
- 2 very elevated IRT levels detected
- 2 elevated IRT results and one or two mutations on DNA analysis
  OR
- Single elevated IRT and one or two mutations on DNA if only one screen received

Case will be handled similar to other newborn conditions:
- Parent will receive a letter with notification of screen results
- PCP will receive a mailer from laboratory with results
- NBS Nurse will call PCP with results and FAX follow up info
- CF center M.D. will be notified of child referred for sweat test

**Sweat Test**

- Gold standard
  - Pilocarpine Iontophoresis
- Collection Methods:
  - Gibson Cook Method
    - Collect sweat on dry gauze with direct measurement of chloride concentration
    - Need 75 mg of sweat
  - Macroduct Coil Collection System with direct measurement of chloride concentration
    - Need 50 mg of sweat

**Abnormal Screen Report**

**Cystic Fibrosis Newborn Screening Algorithm**
Sweat chloride > 60 mEq/L (diagnostic for CF with 1 or 2 CFTR mutations)

Genetic Counseling

Cystic Fibrosis CF Center care

Sweat chloride < 30 mEq/L (normal)

Normal

0 CFTR mutation

1 CFTR mutation

Genetic Counseling

CF Carrier

DSHS Positive CF NBS

Elevated IRT

2 CFTR mutations

Elevated IRT

1 CFTR mutation

Elevated IRT

0 CFTR mutation

CF Center for sweat test

Repeat sweat test +/− Additional mutational analysis (Genzyme or Ambry)

Sweat chloride > 30 but < 60 mEq/L (equivocal results)
Recognize Multi-System Impact of CF

Common Comorbid Conditions in Patients with CF over the Lifespan

Cystic Fibrosis Foundation Patient Registry, 2007

“Adding Tomorrows Every Day”

John Saito, MD, FAAP, FCCP
Cook Children’s Medical Center
Fort Worth, Texas
John.Saito@cookchildrens.org
www.ARTinMEDICINE.com