Overview of CF and CFTR genotyping

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Organ Dysfunction in CF

- **Sinuses** – Sinusitis, nasal polyps
- **Lung** – Endobronchitis, bronchiectasis
- **Pancreas** – Exocrine Insufficiency
  - CF Related Diabetes
- **Intestine** – Meconium ileus
  - Constipation/DIOS
- **Liver** – Focal sclerosis
- **Vas Deferens** – failure to develop
- **Sweat gland** – salt-losing dehydration

Adapted from Welsh and Smith, Sci Am, 1995
Cystic Fibrosis

• Genetic condition – 1/3,500 births; 35,000 individuals in US
• Progressive lung disease

Median FEV$_1$ Percent Predicted vs. Age by Birth Cohort

- Median Predicted Survival:  - 37 years
- Median Age at Death:  - 26 years

FEV$_1$ is steadily improving and stays above 90 percent predicted into adolescence.

Patient Registry, Cystic Fibrosis Foundation, 2008, Bethesda MD, USA (N=c.25,000)
Hypothesis: Improving CFTR function will result in clinical benefit in patients with G551D

First suggested: (Accurso et al, NEJM, 2010, N=39)

Sweat Chloride

Change in sweat chloride concentration (mean, 95% CI)

- Treatment effect through Week 48
  - 48.1 mmol/L
  - *P < 0.0001*

Phase 3 Trial  (Ramsey et al, NEJM, 2011)
Lung Function Improves with VX-770

Treatment effect through Week 48
+ 10.5 %
\[ P < 0.0001 \]
“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 systems should ensure parental and provider education…”
Status of CF NBS in 2004

- **Universally required**
- **Universally offered, but not required**

*Slide courtesy P. Farrell*
Current Status of CF NBS (2007)

- **Universally required**
- **Universally offered, but not required**
- **Offered to select populations or by request**
- **Advanced planning stages**
- **Considering various options**
- **Required but not yet implemented**
- **No information on current intentions**

Slide courtesy P. Farrell
Current Status of CF NBS (2008)

- **Red**: Universally required
- **Orange**: Universally offered, but not required
- **Green**: Required but not yet implemented
- **Yellow**: Advanced planning stages
- **Teal**: Offered to select populations or by request
- **Purple**: Considering various options

*Slide courtesy P. Farrell*
Current Status of CF NBS (2009)

- **Universally required**
- **Approved and scheduled**

12/09?
Current Status of CF NBS (2010)

Universally required

Slide courtesy P. Farrell
Global Distribution of CF Newborn Screening in 2010

Slide courtesy P. Farrell
By 2010, newborn screening was the most common diagnostic indication

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year

Presented at NACFC, November 2011, Anaheim
Age of diagnosis has decreased with newborn screening

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year

Presented at NACFC, November 2011, Anaheim
Complications in US

- U.S. CF Foundation Patient Registry, 2000-2002
- Comparison of
  - Newborn Screening (NBS)
  - Symptomatic Diagnosis (SYMP)
  - Meconium Ileus (MI)
  - Prenatal
- Weight for age
- Height for age
- Hospitalizations
- Pseudomonas aeruginosa infections

Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)
Newborn screened infants were less likely to be malnourished (weight for age < 3\textsuperscript{rd} percentile)

*Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41*
Children with CF who were newborn screened as infants fewer hospitalizations.
Most infants under 2 years in 2010 were diagnosed early
U.S. CF Foundation Registry

- 83% of children < 2 years by the end of 2010 were identified by NBS or MI
- The oldest baby in Texas identified under newborn screening was <1 in 2010 (~400,000 births/year, 60 babies with CF/year)
IRT/IRT

- Newborns receive 2 newborn screen tests
  - 1st before hospital discharge
  - 2nd at 2 week well baby check (mandated or extra sample collected)
- IRT is tested on both newborn screen blood spots
- If both IRTs are elevated, child is recalled for a sweat test (e.g. cutoffs at 100ng/ml and 70ng/ml)
- No genetic testing is performed – no carriers are identified
Introduction of mutation analysis to CF NBS

• CF Mutation identified in 1989
IRT/DNA

- Newborns receive 1 newborn screen tests
- IRT is tested on dried blood spot
- If IRTs is elevated, same sample is tested for CFTR mutations.
- If 1 or more CFTR mutations are identified child is recalled for a sweat test
  - 2 mutations – presumptive positive (sweat test)
  - 1 mutation – possible CF (sweat test)
## Comparisons of Different Screens

<table>
<thead>
<tr>
<th>IRT/IRT</th>
<th>IRT/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tend to be &gt;99th %</td>
<td>Tend to be 96-98 %</td>
</tr>
<tr>
<td>Must wait for 2nd test</td>
<td>Earlier Diagnosis</td>
</tr>
<tr>
<td>No genetic info</td>
<td>Genetic Counseling Required</td>
</tr>
<tr>
<td>IRT Cutoffs</td>
<td>Genetic Results</td>
</tr>
</tbody>
</table>
IRT/IRT$_1$$\uparrow$/DNA

- Decrease 1$^{\text{st}}$ screen cutoff
  - 105ng/ml (99.7 %ile) to 97$^{\text{th}}$ %ile (~55ng/ml)
- **Link** 1$^{\text{st}}$ and 2$^{\text{nd}}$ screen specimens for each baby
- Test 2$^{\text{nd}}$ screen ONLY if first screen $> 97\%$ile
- Mutation analysis if BOTH first and second screen results $> 97\%$
IRT/DNA-EGA

- Newborns receive 1 newborn screen tests
- IRT is tested on dried blood spots
- If IRTs is elevated, same sample is tested for CFTR mutations.
  - If 2 CFTR mutations are identified child is recalled for a sweat test, presumptive positive
  - If 1 CFTR mutation is identified same blood spot tested by expanded genetic analysis methods
    • If additional mutation(s) identified – sweat test
    • If no additional mutation identified – genetic counseling
- Fewer babies recalled for sweat tests
IRT/IRT has the highest sensitivity for the same cutoffs

However the positive predictive value is poor (many more sweat tests)

Sontag et al, J Peds 2009
Goals for NBS Tests in CF

- Minimize false negatives (Sensitivity)
- Balance the number of false positives (PPV)
- Provide a more specific diagnosis, i.e. DNA
- Minimize the need for genetic counseling for detection of carriers
- Reduce parental stress
  - Reduce the time to a diagnosis
  - Reduce the number of children/parents recalled for testing
- Reduce costs of screening and follow-up
Advantages to adding DNA testing to CF NBS

• Offers a more specific result in many cases
  – >60% of CF cases had 2 mutations.

• Can provide additional genetic information
  – Allow genetic counseling of parents of carriers
Challenges to adding DNA testing to CF NBS

• Clinicians ‘trust’ DNA
  – Need to educate clinicians that mistakes can happen in all tests

• Identification of carriers requires counseling

• May miss individuals with rare mutations (especially challenging in Hispanic populations in CF)
Selection of CFTR mutations

• Only mutations known to cause CF should be included in a panel

• 23-mutation ACMG
  – High degree of sensitivity
  – All mutations known to cause disease (special case R117H*)

• Additional mutations added when needed for population coverage for regional differences
<table>
<thead>
<tr>
<th>Allele</th>
<th>CA* (23)</th>
<th>MA* (24)</th>
<th>NY* (24)</th>
<th>CO* (25)</th>
<th>WI* (26)</th>
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<tr>
<td></td>
<td>N=70</td>
<td>N=112</td>
<td>N=108</td>
<td>N=317</td>
<td>N=21</td>
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<tr>
<td>F508</td>
<td>75.3</td>
<td>67.9</td>
<td>57.4</td>
<td>71.3</td>
<td>66.7</td>
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<tr>
<td>G542X</td>
<td>6.2</td>
<td>1.3</td>
<td>3.2</td>
<td>3.8</td>
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<tr>
<td>G551D</td>
<td>3.7</td>
<td>3.1</td>
<td>1.4</td>
<td>1.4</td>
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<tr>
<td>W1282X</td>
<td>3.7</td>
<td>1.8</td>
<td>0.9</td>
<td>1.1</td>
<td>2.4</td>
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<tr>
<td>621+1G&gt;T</td>
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<td>0.4</td>
<td>0.5</td>
<td>1.6</td>
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<tr>
<td>R553X</td>
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<td>0.4</td>
<td>0.9</td>
<td>1.8</td>
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<tr>
<td>3120+1G&gt;A</td>
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<td>0.5</td>
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<td>I507del</td>
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<td>0.5</td>
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<tr>
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<td>3.2</td>
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<tr>
<td>3849+10kbC&gt;T</td>
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<td>0.5</td>
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<td>R334W</td>
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<td>R117H †</td>
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<td>0.9</td>
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<td>‡</td>
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<td>R347P</td>
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* CA = California; MA = Massachusetts; NY = New York; CO = Colorado; WI = Wisconsin.
† Detection of this allele trans to a disease-causing mutation was excluded from percentages reported by these authors, but would have been > 1%.
‡ Not tested in this mutation panel.

CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guidelin.* CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011
Balance of sensitivity/PPV

- Sensitivity: as long as one mutation from an affected patient is on panel, infant will be referred for sweat testing
- PPV: With the inclusion of too many mutations, more carriers will be called back for sweat testing
Detection of CF Cases and Carriers at Different Levels of Mutation Panel Sensitivity

Theoretical Population of 1000 Newborns With High IRT Referred for DNA Testing

CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guidelin.* CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011
Methods used

• Most state labs that are doing multiple CF mutation detection are using:
  – Luminex based assay (all FDA approved)
  – Hologic Inplex assay (ACMG23 FDA approved)
  – ACMG 23
  – ACMG 23 plus additional mutations.
## Reporting of results
### IRT/DNA

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<td>IRT level</td>
<td>CF screen normal</td>
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<tr>
<td>Mutation analysis</td>
<td>No mutations</td>
<td>IRT level No mutations detected</td>
<td>CF screen normal</td>
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<tr>
<td>Mutation analysis</td>
<td>One mutation</td>
<td>IRT level and mutation</td>
<td>Sweat chloride testing</td>
</tr>
<tr>
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<td>Two mutations</td>
<td>IRT level and mutations</td>
<td>Call PCP Sweat chloride testing</td>
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