Application of CFTR Genotype-Phenotype Information to Newborn Screening for CF in California

California Newborn Screening for CF: Genetic Counselors’ Role in Follow-up For Positive CF Results With a Non CF-Causing Mutation

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Three (3) major learning outcomes of the webinar(s):

1. Describe the newborn screening processes for cystic fibrosis in California
2. Identify the variants deemed “non CF-causing” by the CFTR2 project
3. Employ the new California state protocol for screen-positive babies carrying one or more non CF-causing variants

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www.cfri.org

Instructions for claiming CEUs
Self Report Form
Evaluations Form
California Newborn Screening for CF: Genetic Counselors’ Role in Follow-up For Positive CF Results With a Non CF-Causing Mutation

Martin Kharrazi, PhD and Shellye Lessing, MS
Genetic Disease Screening Program
Webinar - 18 JUNE 2014
Outline of Presentation

1. Background on NBS for CF in CA
2. CFTR2 classification of mutations
3. Examples of non CF-causing mutations
4. CA newborn screening experience
5. New GC-Only Follow Up Approach
Background – What is cystic fibrosis?

• CF is an autosomal recessive genetic disorder
• Defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein resulting from a mutation in the CFTR gene
• Symptoms result from faulty transport of chloride and bicarbonate ions through the CFTR across the membranes of cells
• Multiple organs and systems may be affected:
  – Lungs
  – Digestive tract
  – Sinuses
  – Sweat glands
  – Male reproductive system
• Symptoms include:
  – Clearance of lung secretions impeded, causing inflammation and recurrent infection
  – Pancreatic enzymes cannot reach the intestines, causing runny, greasy, smelly stools, malnutrition and poor weight gain and growth
  – Very salty sweat, leading to dehydration and electrolyte imbalance
Typical 2- vs. CA 3-Step CF NBS Model

**Typical 2-Step Model**

- **Step 1:** IRT Testing
  - High IRT (Top 4.0%-5.0%)
  - Step 2: DNA Mutation Panel (1-40 Mutations)
  - 1 or 2 Mutations
  - Low IRT

**California 3-Step Model**

- **Step 1:** IRT Testing
  - High IRT (Top 1.6%)
  - Step 2: Customized DNA Mutation Panel (40 mutations)
  - 2 Mutations
  - 1 Mutation

**Step 3:** DNA Sequencing (<1 per 1000 live births)

- 2 or more Mutations/Variants (35%)
- 1 Mutation
  - Telephone Carrier Counseling (~13%)

**Screen Positive**

**Sweat Test and Clinical Follow up at CF Center**

**Screen Negative**

**Sweat Test at CF Center**

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Current Definition of 2 or More Mutations/Variants

**Step 2:**
2 mutations*

or

1 mutation* and

**Step 3:**
5T variant (TG Tract – 12 or 13)

and/or

Novel variant

and/or

Mutation associated with CFTR-related disorders

and/or

CF-causing mutation

*CA-40 Panel
Statistics from First 6 Years of CA CF NBS
(N=3,065,464 screened births)

- Total CF Cases: 421
- Total CRMS Cases: 539
- Total Carriers: 1919
- Total Missed Cases: 33

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Screening Performance
CA CF NBS Yrs 1-6

• The ratio of screen positives to cases detected is 2.8 to 1. (1,181 screen positives / 421 cases detected)
  – The ratio changed to 2.5 to 1 excluding 115 false screen positives with 1 panel mutation + (TG) 11-5 (T) or I1027T + delF508

• Missed cases: 33 CF cases with negative screening results were reported

• Case detection rate = 92.7%
  – 421 cases detected by screening / 454 total CF cases
# CF Cases with False Negative CF NBS Results, Years 1-6* (N=33)

<table>
<thead>
<tr>
<th>Screening step yielding false negative (reason)</th>
<th>N</th>
<th>Observed IRT levels and genotypes</th>
<th>Race / Ethnicity</th>
<th>CF diagnosis suggested by:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step 1</strong> (IRT below cutoff)</td>
<td>18</td>
<td>IRT (ng/mL): 9, 9, 16, 16, 20, 28, 28, 29, 31, 34, 38, 48, 51, 52, 52, 54, 57, 58&lt;br&gt;Allelic frequency: delF508 (13); unknown (7); L206W (2); R117H (2), 1002-7delTTT, 13TG-5T, 1924del7, 2307insA, 303_304insA, 5′UTR-156G&gt;C, delF311, D836Y, G542X, G576A, R668C, S1235R (1)</td>
<td>W (n=10), H (n=5), Other (n=2), W/B (n=1)</td>
<td>Symptoms (n=11), prenatal diagnosis (n=3), family history (n=1), MI (n=3)</td>
</tr>
<tr>
<td><strong>Step 2</strong> (no mutations on panel)</td>
<td>11</td>
<td>2822delT / 2822delT (n=3); 1288insTA / 1288insTA; 1525-42G&gt;A / 2622+1G&gt;A; 3368-2A&gt;G / 1679+1643G&gt;T; c.1792_1798delAAAACTA / Q890X; 711+5G&gt;A / 1078delT; E1401X / 4279insA, 297-3C&gt;T; EX3del / In18_EX20del; R74W, V201M, D1270N, T854T / 13TG-5T</td>
<td>H (n=8), W/O (n=1), Other (n=2)</td>
<td>MI (n=6), family history (n=2), family history/symptoms (n=2), symptoms (n=1)</td>
</tr>
<tr>
<td><strong>Step 3</strong> (2nd mutation not detected during Step 3)</td>
<td>4</td>
<td>delF508 / duplication of CFTR exons 6b through 10; G551D / CFTRdel22,23; dell507 / none detected by MLPA; delF508/s(TG)11-7T</td>
<td>W (n=3), H (n=1)</td>
<td>MI (n=3), symptoms (n=1)</td>
</tr>
</tbody>
</table>

*7/16/07-6/30/13
Background – What is CFTR1?

CFTR1 (http://www.genet.sickkids.on.ca/app)

- Initiated by the Cystic Fibrosis Genetic Analysis Consortium in 1989
- Maintained by Cystic Fibrosis Centre at the Hospital for Sick Children in Toronto
- Database with up to date, world-wide, information about individual mutations in the CFTR gene
- 1971 CFTR mutations currently listed
- Clinical information relates only to the details of discovery of specific mutations
Welcome to the Clinical and Functional Translation of CFTR (CFTR2) website

CFTR2 is a website designed to provide information about specific cystic fibrosis (CF) mutations to patients, researchers, and the general public. For each mutation included in the database, the website will provide information about:

- Whether the mutation causes cystic fibrosis when combined with another CF-causing mutation, and
- Information about the sweat chloride, lung function, pancreatic status, and pseudomonas infection rates in patients in the CFTR2 database with this mutation.

Information on the CFTR2 website is being updated as further analysis is completed. The most up-to-date clinical information and results of functional testing are available on individual mutation pages. For a complete list of CFTR2 mutations and their characterizations, please visit CFTR2 Mutation List History (available under the Quick Links menu).

For patients and family members

This website provides information about specific CF mutations only. This website is intended for members of the general public who want to find out what we currently know about specific mutations related to cystic fibrosis.

This includes:
- Cystic fibrosis (CF) patients,
- Family members of CF patients,
- People who are carriers of a CF mutation, and
- Parents whose baby has just been diagnosed with CF through newborn screening.

For health care providers/scientists

This section provides scientific and medical descriptions, intended for CF researchers, health professionals, and members of the general public that are looking for more in-depth, research-related information. Patients and their families are encouraged to visit the section "For patients and family members" first.

WHAT THIS SITE IS NOT INTENDED TO DO:

- This website is not intended to help diagnose anyone with CF.

For more information about CF, click here.

Note: "If you have questions about any of the information contained in this website, please consult your doctor."
CFTR2.org mutation reports

- Clinical Characteristics
  - Sweat Chloride Testing Results
  - Meconium Ileus
  - Pancreatic Insufficiency
- Mutation Characteristics
  - Stop Codon
  - Missense
- Functional Testing
  - Chloride conductance in cell based systems
- Literature Review
  - Studies of homozygotes
- Population Screening
  - Penetrance studies
- Bioinformatics Assessment
  - Machine learning in silico approach for rare mutations
CFTR2 mutation categories

1. CF-causing
2. Varying clinical consequence
3. Non CF-causing
4. Unknown significance

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Non CF-causing CFTR2 mutations – 3 types

1. Mutation variants only found in *cis* phase with known CF-causing mutations
   - I1027T is only found in *cis* with F508del

2. Mutation variants that only cause CF when found in *cis* phase with another mutation variant (complex mutation)
   - Poly 5T with preceding 13 vs. 11 TG repeats

3. Non CF-causing mutations known to be in *trans* phase with CF-causing mutations
Number of Screen Positives with 7 CFTR2 non CF-causing mutations*

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Total Newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td>L997F</td>
<td>63</td>
</tr>
<tr>
<td>R1162L</td>
<td>13</td>
</tr>
<tr>
<td>R668C + G576A</td>
<td>13</td>
</tr>
<tr>
<td>S1235R</td>
<td>12</td>
</tr>
<tr>
<td>R31C</td>
<td>9</td>
</tr>
<tr>
<td>R668C only</td>
<td>5</td>
</tr>
<tr>
<td>V754M</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>119 (9%)</strong></td>
</tr>
</tbody>
</table>

* Among 1323 CF NBS screen positive newborns in CA (as of 5/13/2014)
7 CFTR2 non CF-causing mutations by *trans* phase*

<table>
<thead>
<tr>
<th>Non CF-causing Mutation</th>
<th>Panel Mutation</th>
<th>Total Newborns</th>
<th>% in <em>trans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>L997F</td>
<td>2055del9&gt;A; 3849+10kbC&gt;T; 3876delA; delF311; delF508; delI507; G542X; W1089X</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>R1162L</td>
<td>1717-1G&gt;A; 3120+1G&gt;A; delF508; G542X; P205S</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>R668C + G576A</td>
<td>delF508; G542X; S549N; W1089X</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>S1235R</td>
<td>3120+1G&gt;A; 3849+10kbC&gt;T; delF508; W1282X</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>R31C</td>
<td>delF508; G551D</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>V754M</td>
<td>2105-2117del13insAGAAA; 406-1G&gt;A; delF508</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>R668C only</td>
<td>delF508</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>R668C only</td>
<td>3849+10kbC&gt;T</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>71</strong></td>
<td><strong>98.6</strong></td>
</tr>
</tbody>
</table>

* Determined by parent mutation testing out of a total of 119 newborns with one of the 7 CFTR2 non CF-causing mutations.
L997F  (Source: CFTR2.org Summary)

**Summary:** L997F 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.

L997F is an example of a complex allele (see Glossary).
# L997F - CA data (N=34)

<table>
<thead>
<tr>
<th><strong>Patient Descriptors</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age</td>
<td>2 to 5 ½ years</td>
</tr>
<tr>
<td>Status</td>
<td>56% active, 38% inactive*</td>
</tr>
<tr>
<td>Gender</td>
<td>53% female</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>73% Hispanic, 26% Non Hispanic White</td>
</tr>
</tbody>
</table>

## Outcomes

<table>
<thead>
<tr>
<th><strong>Meconium Ileus</strong></th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest sweat value (age)</td>
<td>20.4 ± 8 mEq/L (1 ½ m to 5 y)</td>
</tr>
<tr>
<td>PI status on Ann. Pt. Summary</td>
<td>2</td>
</tr>
<tr>
<td>Pancreatic enzymes</td>
<td>6 (FE values all &gt;200 mcg/g)</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>N=3, once in each</td>
</tr>
<tr>
<td><strong>Anti-PSA treatments</strong></td>
<td>none</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>N=1</td>
</tr>
<tr>
<td>Other microorganisms</td>
<td>MSSA, Candida, Streptococcus</td>
</tr>
</tbody>
</table>

*Inactive status = Lost to follow up, treatment deemed unnecessary, and parental refusal.

Slide courtesy of Danieli Salinas (CHLA)
Uncertainties

• Will multi-organ CF develop over time?
• Will single organ CFTR-related disease manifest over time?
• Will males be infertile?
• If so, will prompt delivery of care at a CF Center minimize the health effects?
Challenges

• What type of referral after NBS is best for patients with one of these seven mutations?
• Educating parents about uncertainties without creating stress and psychosocial effects
Follow-up Changes Recommended by California CF NBS Consortium

- Full diagnostic workup not needed unless symptomatic or positive family history for CF
  - Sweat tests have been normal in newborns & infants
  - No failure to thrive or persistent lung symptoms documented in infancy
Follow-up Recommendations, Cont.

• Because of possible later single system CFTR-related symptoms, these patients should not be regarded simply as carriers.

• The discussion of the results and implications is complex and best done:
  – In person
  – In a neutral setting that does not convey that the child has a medical condition
Follow-up Recommendations, Cont.

– By a professional trained in helping families deal with the uncertainties of the genetic test results and how health may or may not be affected by certain genotypes

• Conclusion: Genetic Counselors are uniquely qualified to be the primary providers of information to assist families in understanding the implications of the newborn’s genotype

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Modified CA 3-Step CF NBS Model

Screen Positive

Screen Negative

Screen Positive – modified protocol for 7 CFTR2 non CF-causing mutations

Step 1: IRT Testing

High IRT (Top 1.6%)

Low IRT

Step 2: Customized DNA Mutation Panel (40 mutations)

2 Mutations

1 Mutation

0 Mutations

Step 3: DNA Sequencing

2 or more Mutations/Variants

1 Mutation

Telephone Carrier Counseling

Sweat Test and Clinical Follow up at CF Center

Genetic Counseling Only

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Follow-up Recommendations, Cont.

• Refer as CF screen positive to a CF Center to schedule genetic counseling as the recommended follow-up

• Consider CF Center evaluation and possible sweat testing if indicated by symptoms, family history
Follow-up Process

NBS ASC → Call w Positive → PCP

Genetic Counseling → Parents

Parents → CF Center

1. Consult

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Follow-up Changes Recommended by California CF NBS Consortium

- Family receives information about:
  - The specific mutations in the baby and the genetics of CF
  - Possible signs or symptoms of CFTR dysfunction to watch for
  - CF Center as an important future resource if symptoms develop
Interpretation on Newborn Screening CFTR DNA Sequencing Results Mailer

• “This child carries one severe CF mutation and one sequence change of unknown clinical significance. Consultation by the PCP with a CCS approved CF Center is required prior to discussion with the family.”

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Follow-up Responsibilities

NBS Area Service Center Coordinators
• Cases appear on daily report as CF Positive for follow-up
• Contact Primary Care Provider (PCP) with results, determine CF Center for follow-up
• Fax information sheet to PCP
• Follow case until genetic counseling is completed
PCP Fact Sheet

• This child was found to have one disease-causing CF mutation and another CFTR mutation of unknown clinical significance.

• *There is new research information about the mutation of unknown clinical significance.* Before contacting the family with the screening results, please **call ______ CF Center at (phone #)** to discuss this new research so you can provide the family with up-to-date information.
Based on this research, the child is very unlikely to exhibit the multiple clinical signs and symptoms that are common with CF such as
- elevated sweat chloride and low fecal elastase values
- chronic cough, sinusitis and respiratory illness
- slow growth, pancreatitis and digestive problems
- in males, congenital bilateral absence of the vas deferens.

As the child ages, however, his/her risk to develop any single clinical sign or symptom may increase.
PCP Fact Sheet

• The child should be referred for genetic counseling at a CF specialty care center to discuss the specific CFTR mutations identified in their child’s newborn screen, the genetics of CF, and to learn about the CF-related symptoms to watch for.

• In the future, if the child ever exhibits any of the CF-related clinical signs and symptoms listed above, please immediately refer her/him to a CF specialty care center for evaluation and treatment.
Follow-up Responsibilities

CF Centers

• Receive the referral, provide consultation with the PCP regarding the screen positive NBS results
• Schedule/assist in referral for genetic counseling in CF Center or Medical Center
• If there are causes for concern for the center staff or family, further follow-up such as a clinical examination or sweat testing may be done
Follow-up Responsibilities

Genetic Counselor

• Receive the referral with the NBS CFTR mutation results & clinical significance from the CF Center

• One CF-causing (panel) mutation and one of the following CFTR Mutations: G576A + R668C, L997F, R31C, R668C, R1162L, S1235R, V754M was identified

• Provide genetic counseling for parents of the newborn – for consistency in counseling, a GC Checklist for information to be provided has been developed
Genetic Counseling Guidelines: NBS Positive for One CF Mutation and One Non CF-Causing Mutation

• Review how NBS testing is done for CF and which mutations were found for the baby

• Reassure parents that the baby is not expected to have the multiple clinical signs and symptoms of CF

• If asymptomatic, no sweat test or treatment needed
Genetic Counseling

NBS CF Test Limitations, CF Symptoms

• Discuss limitations of testing (it’s rare, but an additional mutation could be missed by the test method; if so, multiple symptoms could occur)

• Review symptoms of multi-system CF
  – Poor weight gain, digestive problems
  – Chronic sinusitis, cough or respiratory illness
  – Pancreatitis
  – Frequent loose, oily, foul smelling stools; stomach aches
  – Extra salty sweat (residue of sweat on clothes)
Genetic Counseling

Baby’s NBS Results Implications

• Explain that the child may have a chance to develop any single CF-related symptom later in life (studies continue on how likely this is)
  – chronic cough or sinus infections
  – pancreatitis
  – for males, infertility due to congenital bilateral absence of the vas deferens (CBAVD)
Genetic Counseling

When to Contact CF Center, Family History

• Recommend that if, in the future, the child has any persistent symptoms described above, the parents contact the primary care provider and the CF Center for evaluation, testing and treatment.

• Obtain family history of CF, CF carriers, CF symptoms; discuss inheritance pattern
Genetic Counseling

Dealing With Uncertainty

• Assist family in dealing with uncertainty about the significance of the CFTR mutations. An analogy might be personal genomic testing that reveals an increased chance for a certain condition.
Genetic Counseling

Future Pregnancies

• Discuss CF carrier testing for the parents if planning a future pregnancy.
  – Currently GDSP does not pay for parent testing
  – **Note:** the parent who carries the non CF-causing mutation could have a CF-causing mutation on the other chromosome and be asymptomatic. In this case a future child would have a 25% chance of inheriting a CF-causing mutation from each parent. A mutation panel containing known CF-causing mutations and appropriate for ethnicity is recommended. CFTR DNA sequencing is not necessary.

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Genetic Counseling

Family CFTR Carrier Testing

- If parent testing has not been done and sibling or extended family testing is requested, consider recommending a lab that can do gene sequencing or targeted gene sequencing to detect the uncommon mutation.
- Provide information/referral for prenatal genetic counseling services as appropriate.
Genetic Counseling
Summary letter

• Provide a letter to the family with copy to PCP (template from GDSP) summarizing the information provided in the counseling session with:
  – infant’s CFTR mutations, implications for health
  – list of symptoms to contact the CF Center about
  – CF Center contact information
Future Expectations

• There will be many other CFTR mutations that will likely fall into the non CF-causing category

• Post NBS screening approach to referral and/or education will be critical to get right as we enter into the new world of personalized medicine and newborn genomic sequencing
Acknowledgements

• **Cystic Fibrosis Research, Inc.** [WWW.CFRI.org](http://WWW.CFRI.org) - David Soohoo and Sue Landgraf, for technical support and hosting the slides, webinar recording and CEU information for genetic counselors on their website.

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• **California Genetic Disease Screening Program** – Michet Sylvester and Irene Mandujano for technical support

• **California CF Newborn Screening Consortium**
RESOURCES

- **California Newborn Screening Program**
  [www.cdph.ca.gov/NBS](http://www.cdph.ca.gov/NBS) Information on CF and NBS for parents and providers, listing of California Children’s Services-Approved Cystic Fibrosis Special Care Centers

- **CFTR2 Project**: [www.CFTR2.org](http://www.CFTR2.org)

- **Contact us**: Marty.Kharrazi@cdph.ca.gov
  Shellye.Lessing@cdph.ca.gov

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