Overview of the First Eight Years Of Newborn Screening For Cystic Fibrosis: The California Experience.

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Newborn Screening for Cystic Fibrosis in California

- In July 2006 legislation passed that added Cystic Fibrosis to the Newborn Screening Panel in California.
CF Newborn Screening Development

California faced specific challenges in implementing NBS for CF including:

• The large number of births (500,000/year).
• A poor understanding of the common CFTR mutations in our very heterogeneous population.
• Limited resources at CF Centers for all the positive tests that would result if we followed the standard IRT/DNA model.
CF Newborn Screening Development

• Our goals were:
  – Maximize case detection
  – Minimize the burden of false positives, false negatives, sweat chloride tests and the associated follow-up costs
  – Identify at least 90% of all Hispanic, non-Hispanic White and Black children with CF

• A detailed study of the three main race/ethnic groups was carried out to determine the most common mutations.

• A 3-step model of immunoreactive trypsinogen (IRT)-mutation panel- DNA sequencing model for CF newborn screening was developed that would work for our program.
California 3 Step CF NBS Model

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 (<1 per 1000 live births)
- 2 or more Mutations/Variants (35%)
- 1 Mutation
  - Telephone Carrier Counseling (~13%)

Sweat Test and Clinical Follow up at CF Center
Screening Results/Data
Program Data Summary

Of 4,051,563 births in CA over the 8 years we have found:

• 502 cases of CF
  – 285 by the panel (100%)
  – 217 by sequencing (17% of sequenced)

• 820 CRMS Cases

• 2581 Carriers
368 unique mutations/variants were found in newborns with positive DNA sequencing results, including 38 panel mutations.

200 mutations/variants were seen only once and 111 were novel.

Of the 111 newborns with novel mutations detected, 23 (21%) were diagnosed with CF.

Of cases diagnosed with CF by DNA sequencing, only about half have an initial positive sweat test.

The most common mutations associated with this ‘progression’ from CRMS to CF were IVS8 (TG)13-5T, R117H-7T and D1152H.
## Estimated CF Prevalence by Race/Ethnicity

<table>
<thead>
<tr>
<th>Client Race/Ethnicity</th>
<th>Live births screened</th>
<th>Screen positive</th>
<th>Diagnosed CF</th>
<th>Missed CF</th>
<th>Prevalence 1/X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic/Latino</td>
<td>1,664,800</td>
<td>510</td>
<td>153</td>
<td>14</td>
<td>9,969</td>
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<tr>
<td>Non-Hispanic White</td>
<td>1,035,354</td>
<td>632</td>
<td>231</td>
<td>9</td>
<td>4,314</td>
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<tr>
<td>Non-Hispanic Asian</td>
<td>383,058</td>
<td>23</td>
<td>4</td>
<td>3</td>
<td>54,723</td>
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<tr>
<td>Non-Hispanic Black</td>
<td>211,199</td>
<td>97</td>
<td>18</td>
<td>1</td>
<td>11,116</td>
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<tr>
<td>Other /Multiple</td>
<td>757,152</td>
<td>278</td>
<td>96</td>
<td>12</td>
<td>7,011</td>
</tr>
<tr>
<td>Total</td>
<td>4,051,563</td>
<td>1,540</td>
<td>502</td>
<td>39</td>
<td>7,489</td>
</tr>
</tbody>
</table>
Modifications to Screening Algorithm
IVS8 Poly 5T/TG

- At the start of the program we initially called out all IVS8 Poly 5T with an 11, 12 or 13 TG tract
- After approximately 3 years we had a large number of 11TG-5T (over 100) and follow-up data indicated that they nearly all had negative sweat tests and no symptoms.
- Additional literature was published that confirmed these findings and reported the primary possible concern is congenital bilateral absence of the vas deferens (CBAVD).
- On June 1, 2011 we discontinued calling 11TG-5T as positive. They are now considered ‘carriers’ but there is a small chance of CBAVD in males with 11TG-5T.
CFTR2 ‘Non-CF Causing Mutations’

- Based on John Hopkins-based CFTR2, there is a group of mutations called out by the California program that CFTR2 describes as ‘non CF causing’.

- The mutations that CFTR2 updated are: R668C, G576A, G576A + R668C, L997F, R31C, R1162L, V754M, S1235R.

- On May 2014: continue to refer these children to the CF centers as CF screen positive, but recommend genetic counseling as the minimum follow-up.
• In Spring 2015 we stopped reporting out 1525-42G>A as a mutation through sequencing because newborns/infants with this mutation have had negative sweat tests and done well.

• This mutation is very common in Hispanics and is now classified as a benign polymorphism.
  – was detected in 4.3% of Hispanics in a study by Shrijver et al 2015.
  – Consistent with our findings of a frequency of 1.88% in the CA population as a whole and 4.94% in the Hispanic population.
Screening Performance
Median Age at Key Screening Points for Screen Positive Cases: July 16, 2007–June 30, 2015

Key time points:
- Age at IRT test result
- Age at panel result
- Age at sequencing result
- Age at first sweat test
- Age at diagnosis
- Age at first referral
- Age at first evaluation
- Age at first treatment initiation

 Median age (days):
- Panel (N=285)
- Sequencing (N=1255)
- Total (N=1540)
Screening Performance - Detection

• The ratio of screen positives to cases detected is 3.1 to 1. (1,540 screen positives / 502 cases detected)

• Case detection rate = 92.8%
  – 502 cases detected by screening /541 total CF cases

• Missed cases: 39 CF cases with negative screening results were reported
  – 19 by low IRT
  – 13 by negative panel
  – 7 had negative sequencing
Lessons Learned

• Including sequencing increases the time for result reporting.
• DNA Testing leads to a group who are found to have at least two CFTR mutations or variations but display no symptoms. In California we call this group CRMS.
• Even with sequencing, there are gross deletions or duplications that are not picked up as well as possible lab errors.
• It is very important that physicians know to make referrals if there are signs and symptoms consistent with CF – even with a negative screen.
In Summary

• Including sequencing greatly reduces the false positive rate.
• Sequencing provides more information for the medical team for those who are referred to a CF center.
• We have evidence that a single initial sweat chloride test may not be the best diagnostic ‘gold standard’
  – 43% of diagnosed cases by sequencing did not have an initial positive sweat test (>60 mmol/L).
Acknowledgements - Thanks

- GDSP Program Evaluation Staff
- The California CF Consortium & the staff at the CF centers!
Questions
## CA CF 40 Mutation Panel

<table>
<thead>
<tr>
<th>#</th>
<th>Mutation</th>
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<th>Mutation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1288insTA</td>
<td>21*</td>
<td>del507</td>
</tr>
<tr>
<td>2*</td>
<td>1717-1G&gt;A</td>
<td>22</td>
<td>G330X</td>
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<td>3</td>
<td>1812-1G&gt;A</td>
<td>23*</td>
<td>G542X</td>
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<tr>
<td>4</td>
<td>2055del9&gt;A</td>
<td>24*</td>
<td>G551D</td>
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<tr>
<td>5</td>
<td>2105-2117del13insAGAAA</td>
<td>25*</td>
<td>G85E</td>
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<tr>
<td>6</td>
<td>2307insA</td>
<td>26</td>
<td>H199Y</td>
</tr>
<tr>
<td>7*</td>
<td>3120+1G&gt;A</td>
<td>27*</td>
<td>N1303K</td>
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<tr>
<td>8</td>
<td>3272-26A&gt;G</td>
<td>28</td>
<td>P205S</td>
</tr>
<tr>
<td>9</td>
<td>3791delC</td>
<td>29</td>
<td>Q98R</td>
</tr>
<tr>
<td>10*</td>
<td>3849+10kbC&gt;T</td>
<td>30</td>
<td>R1066C</td>
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<tr>
<td>11</td>
<td>3876delA</td>
<td>31*</td>
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<tr>
<td>12</td>
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<td>32*</td>
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<td>13*</td>
<td>621+1G&gt;T</td>
<td>33*</td>
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<tr>
<td>14</td>
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<tr>
<td>15*</td>
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<td>16</td>
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<tr>
<td>18</td>
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<td>W1204X (3743G&gt;A)</td>
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<td>19</td>
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<td>20*</td>
<td>delF508</td>
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<td>W1282X</td>
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* Mutation included on ACMG 23 Panel