Performance of a three-tier (IRT-DNA-IRT) Cystic Fibrosis screening algorithm in British Columbia

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Issues of Geography

Illinois = 85 people/km²
BC = 5 people/km²

http://www.env.gov.bc.ca/soe/indicators/sustainability/bc-population.html

http://mapfrappe.com
Conflicts of Interest

• None to declare
1. Discuss the challenges of CF screening and diagnosis
2. Compare the BC CF screening algorithm to other approaches
3. Review the outcomes of CF screening in BC
4. Present future considerations
CF Screening Algorithms

IRT (PPV=0.8%)

Steps to deal with the low specificity of the IRT

Sweat Test

IRT

CFTR Panel

Sweat Test

IRT

CFTR Panel

Fail Safe

Sweat Test

IRT

CFTR Panel

Sweat Test

IRT

CFTR Panel

Sweat Test

IRT

CFTR Sequencing

Sweat Test

IRT

CFTR Panel

Fail Safe

Sweat Test

IRT

CFTR Panel

Sweat Test

IRT

Fail Safe

Sweat Test

IRT

Sweat Test

IRT

Sweat Test

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Sweat Test
## IRT-IRT vs IRT-DNA

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Bloodspot</td>
<td>CFTR Test Expensive</td>
</tr>
<tr>
<td>CFTR can be diagnostic</td>
<td>Carrier Detection</td>
</tr>
<tr>
<td></td>
<td>High Sweat test rate</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT is inexpensive</td>
<td>Two samples required</td>
</tr>
<tr>
<td>Reduced Sweat test rate</td>
<td>IRT specificity issues</td>
</tr>
<tr>
<td>Reduced carrier detection</td>
<td></td>
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</tbody>
</table>
### Case Definitions

**CF Foundation Consensus Guidelines** *(Farrell et al 2017)*

<table>
<thead>
<tr>
<th>Condition</th>
<th>CFTR (based on CFTR2.0)</th>
<th>Sweat Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>1 or 2 CF-causing mutations</td>
<td>&gt;60</td>
</tr>
<tr>
<td>CFSPID</td>
<td>2 mutations (&lt;2 CF-causing)</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td>&lt;2 mutations</td>
<td>30-60</td>
</tr>
<tr>
<td>CRMS</td>
<td>2 mutations</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td>&lt;2 mutations</td>
<td>30-60</td>
</tr>
</tbody>
</table>

**CF** = Cystic Fibrosis  
**CFSPID** = CF Screen Positive Inconclusive Diagnosis  
**CRMS** = CF Related Metabolic Syndrome
If second IRT elevated
- CF = 32%
- CFSPID = 12%
- Referred for sweat

If second IRT Normal
- CF = 0%
- CFSPID = 0.3%
- Optional Sweat test
  - Uptake 13%
Positive Outcomes

N=401,977 over 9 years

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>CF +CFSPID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cases</td>
<td>77 (1/5220)</td>
<td>102 (1/3940)</td>
</tr>
<tr>
<td>PPV 2 mut</td>
<td>74%</td>
<td>100%</td>
</tr>
<tr>
<td>PPV 1 mut + High IRT2</td>
<td>32%</td>
<td>44%</td>
</tr>
<tr>
<td>PPV 1 mut + Normal IRT2</td>
<td>0%</td>
<td>0.3%</td>
</tr>
<tr>
<td>PPV V. high IRT + High IRT2</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

1036 sweat tests avoided (78% reduction)
## Negative Outcomes

<table>
<thead>
<tr>
<th>False Negative</th>
<th>CF (% of cases)</th>
<th>CFSPID (% of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IRT1</td>
<td>4 (5.2%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>0 CFTR Mut.</td>
<td>2 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>1 Mut., Normal IRT2</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>1 Mut., High IRT2, Normal Sweat</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>6 (7.8%)</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>

Initial IRT Cutoff = 97th %ile or > 60 ng/ml
  - Missed cases IRT = 21-42 ng/ml (59th-94th %ile)

Mutation Panel
  - 2009-2010 = 23 mutations (Luminex)
  - 2010-2015 = 38 mutations (Third Wave)
  - 2016-Current = 130 mutations (Illumina NGS)
  - 1 of 2 missed cases would be detected by current panel

877 carriers detected
Optional Sweat testing

• 880 apparent carriers (normal IRT2) given option to come to Vancouver for a sweat test.
  – 13% uptake (115)
  – 2 CFSPID cases confirmed (no CF)

• Reasons for opting in:
  – NOT geography
  – First Child
  – Parental anxiety (residual risk of CF)

http://www.env.gov.bc.ca/soe/indicators/sustainability/bc-population.html
• Most attempts to reduce False Negative enrich for CFSPID cases.
  – Lowering initial IRT cutoff
    • Mean CF IRT1 = 144 ug/L
    • Mean CFSPID IRT1 = 77 ug/L
  – Expanded CFTR analysis
    • BC CF:CFSPID = 3.1:1 (130 mutations)
    • California CF:CFSPID = 2:3 (Third tier extended sequencing)

• Implications of CFSPID
  – 5-15% of CFSPID cases convert to CF over time
    • Changes in CFTR2 mutation definition
    • Changes in sweat results

Next Generation Sequencing

• Implemented Illumina CF NGS Kit in 2016
  – MiSeqDx 139 CF-causing mutations
  – Only 1 of 2 missed cases would be picked up with this expanded panel
  – Option to include extended mutation panel
    • all of CFTR 2 as a lab developed assay
    • Concerns:
      – diminishing returns for CF
      – enrichment for CFSPID

https://www.illumina.com/systems/sequencing-platforms/miseq.html
Conclusions

1. An IRT-DNA-IRT protocol significantly reduces sweat test numbers (78% reduction)
2. High PPV for carriers with high IRT2 = 32%
   – Negative PV of normal IRT2 is near 100%
3. The “fail safe” arm has near zero PPV for CF
4. Initial IRT remains the main cause of false negatives
Acknowledgements

- BC Newborn Screening Program
  - Hilary Vallance (Medical Director)
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- BC CF NBS Team
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