Implement New Technologies in NBS: Next Generation Sequencing in NBS for Cystic Fibrosis

**Mei Baker, MD, FACMG**

Co-Director, Newborn Screening Laboratory at WSLH  
Professor, Department of Pediatrics  
University of Wisconsin School of Medicine and Public Health

Gene Sequencing in Public Health Newborn Screening Meeting  
Atlanta, GA  
February 16-17, 2017

**Robert Guthrie, MD, PhD (1916-1995)**

Advances in technology and methodology have often driven improved screening procedures!!!
### Progression of CF NBS Tests

IRT → IRT/DNA → IRT/DNA* → IRT/DNA/DNA**
(F508del) (CFTR-23) (CFTR > 200)

1979 → 1991 → 2003 → 2012-16

*With IRT/DNA, 10 heterozygote carriers are detected for every CF infant diagnosed.

**IRT/NGS algorithm applying CFTR2 knowledge and next generation sequencing capability, which may be a “game-changer.”

### CFTR2 Mutation List History

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35,312</td>
<td>39,696</td>
<td>39,696</td>
<td>88,664</td>
<td>88,664</td>
</tr>
<tr>
<td></td>
<td>CF-causing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>123</td>
<td>175</td>
<td>179</td>
<td>242</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td>Varying Clinical Consequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Non CF-causing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Unknown Significance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>158</td>
<td>203</td>
<td>207</td>
<td>276</td>
</tr>
</tbody>
</table>
IRT/NGS in NBS for CF

IRT

NGS CFTR mutations panel

One Mut.

Screening Normal ??

Two Mut.

Screening Positive

Technical Feasibility Study

Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study

Mei W. Baker, MD1, Anna E. Akcins, MPH1, Suzanne K. Cordovado, PhD1, Mylene Hendria, MS2, Marie C. Zunie, PhD1 and Phillip M. Farrell, MD, PhD1

Purpose: Many regions have implemented newborn screening (NBS) for cystic fibrosis (CF) using a limited panel of cystic fibrosis transmembrane regulator (CFTR) mutations after implementation of new screening using next-generation sequencing (NGS) technology.

Methods: A NGS assay was used to screen 165 CFTR mutations/variants characterized in the CFTR2 project, followed by direct blood spot (DBS) testing and 44 variants of the mutations validated in the NGS assay was successfully performed as 165-CF NGS screen-positive samples with one CFTR mutation.

Results: The NGS assay was successfully performed using DNA isolated from DBS, and 165 screen-positive infants with one CFTR mutation, 10 additional disease-causing mutations were identified in 135 samples containing a second variant. Five infants had a C508T mutation that was not included in this panel, and nine without one CF-causing mutation were identified.

Conclusion: The NGS assay was 99% concordance with traditional methods. A prospective analysis results indicates that the NGS assay allows for an early detection of positive predictive value (PPV). This study lays the foundation for further studies and for implementing NGS in newborn screening.

Key Words: cystic fibrosis, newborn screening, cystic fibrosis transmembrane conductance regulator, next-generation sequencing, newborn screening.
NGS CF Assay

- A validated assay using newborn screening dried blood specimens
- Cover all CFTR exons and known intronic CF causing mutations
- Include Ex2-3 and Ex22-23 deletion
- Simultaneously detect all mutations in a pre-determined panel

Prospective Study

- **SPECIFIC AIM 1.** We will further modify the established Illumina NGS method to expand CFTR mutation panel up to 250 CF-causing mutations.

- **SPECIFIC AIM 2.** We will demonstrate that the IRT/NGS CF screening protocol can significantly reduce false positive results caused by identification of CF heterozygote carrier infants in a real-world NBS environment.
Screening Flowchart
(Incorporating NGS)

WISCONSIN STATE LABORATORY OF HYGIENE - UNIVERSITY OF WISCONSIN

IRT (top 4%, daily)

Next Gen Sequencing

ACMG 23 Mutation Panel

Results Reported; note indicating NGS result to follow

Results Reported and sweat test requested; note indicating NGS result to follow

Abnormal Report / Call to Physician

Results to PCP and sweat test requested; call to physician

Normal Report

PRELIMINARY REPORT

FINAL REPORT

RESULTS REPORTED

PRELIMINARY REPORT

FINAL REPORT

Screening Flowchart
(NGS only since April 1, 2016)

WISCONSIN STATE LABORATORY OF HYGIENE - UNIVERSITY OF WISCONSIN

IRT (top 4%, daily)

Next Gen Sequencing

Two Mutations

One Mutation

No Mutations

Two Mutations

One Mutation

No Mutations

Results Reported; note indicating NGS result to follow

Results Reported and sweat test requested; note indicating NGS result to follow

Abnormal Report / Call to Physician

Results to PCP and sweat test requested; call to physician

Normal Report
Updated IRT/NGS in NBS for CF

IRT

NGS CFTR Mutations Panel (CF Causing Mutations)

Screening Positive

Two Mut. One Mut.

Screening Normal

No Mut.

NGS CFTR Mutations Panel (MVCC Mutations)

Screening Positive

The Future in CF Diagnosis and Treatment:
NBS with more CFTR mutations; better education; better communications; miraculous CFTR modulator therapies
NGS ➔ WGS/WES

NGS in NBS—Technology aspect
- Capacity and flexibility
  - WGS
  - WES
  - Targeted diseases/genes
  - Targeted analysis
- Data storage and management
- Data interpretation
- Data reporting
- Ethical and autonomy issues (genomic technology)

NGS in NBS—Program aspect
- First tier testing for some conditions
- Multiplexing
  - Multiple diseases
  - Multiple genes
- Turnaround time
- Carrier detection
- Unknown clinical consequence mutations
- Cost
  - NBS testing vs. NBS system

Summary
- Clinical utility
  - Establish capability to screen for new disorders
  - Improve screening performance
- Technical feasibility
  - Applicable to DBS
  - Turnaround time
  - High throughput
- Mutation databases and references
- Method selection
- Cost consideration
- Assay validation
  - Qualitative assay vs. quantitative assay
  - Accuracy, precision, reproducibility, linearity, population normal range, clinical validity
- QA and QC consideration
- Results interpreting and reporting
Funding Support

The Legacy of Angels Foundation

Co-founders: Paul and Sue Rosenau

Acknowledgments

- **CF IRT/NGS Laboratory Testing**
  - Sean Mochal, BS (WSLH)
  - John Moon, MS (WSLH)
  - Michael Cogley, BS (WSLH)
  - Sam Dawe, PhD (WSLH)
  - Anne Atkins, MPH
  - Rachel Morgan, BS

- **CF IRT/NGS Investigators**
  - Philip Farrell, MD, PhD (UWSMPH)
  - Michael Rock, MD (UWSMPH)
  - Peggy Modaff, CGC (UWSMPH)
  - Anita Laxova, BS (UWSMPH)