The Role of Targeted Gene Sequencing in U.S. Newborn Screening Laboratories

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2017 APHL Annual Meeting & Eleventh Government Environmental Laboratory Conference
June 12, 2017
Gene Sequencing Timeline in Routine Newborn Screening

- **2004**
  - Hemoglobinopathies
  - Texas begins *HBB* sequencing 3rd tier test

- **2005**
  - Cystic Fibrosis
  - CA begins *CFTR* sequencing 3rd tier

- **2006**
  - Krabbe
  - NY begins *GALC* sequencing 2nd tier

- **2007**
  - Advent of Next Gen Sequencing
  - X-ALD
  - NY begins *ABCD1* sequencing 3rd tier

- **2008 - 2012**
  - APS Creates Molecular Subcommittee for NBS

- **2013**
  - NY begins *GAA* sequencing 2nd tier
  - X-ALD
  - CA begins *ABCD1* sequencing 3rd tier

- **2014**
  - Wisconsin begins *CFTR* Next Gen Sequencing/Genotyping 2nd tier

- **2015**
  - Kansas begins *CFTR* Next Gen Sequencing/Genotyping 2nd tier

- **2016**
  - New Eng: 2nd tier
  - Pompe & MPS 1 (*GALC, GAA & IDUA*)
  - MN: 2nd tier
  - WI: 2nd tier
  - VLCAD (*ACADVL*)

- **2017**
  - 2004
  - Hemoglobinopathies
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    - New Eng: 2nd tier
    - Pompe & MPS 1 (*GALC, GAA & IDUA*)
    - MN: 2nd tier
    - WI: 2nd tier
    - VLCAD (*ACADVL*)

  - **2017**
    - MPS 1 (*IDUA*)
    - NY: 2nd tier
    - Krabbe, Pompe & MPS 1 (*GALC, GAA & IDUA*)
    - NJ: 2nd tier
    - Pompe, MPS 1, X-ALD (*GAA, IDUA, & ABCD1*)
    - New Eng: 2nd tier
    - Pompe & MPS 1 (*GAA, IDUA*)
    - MN: 2nd tier
    - Pompe (*GAA*)
    - WI: 2nd tier
    - VLCAD (*ACADVL*)
    - TX: 2nd tier
    - Cystic Fibrosis Next Gen Seq
    - NY: 3rd tier
Reasons NBS Programs Choose Sanger or Next Gen Sequencing

- Gene Size
- Throughput
- Turnaround time

Sample Preparation – Library Construction
- Next Generation sequencing: 6 to 8 hours
- Sanger sequencing: 1 to 2 hours pending number of samples and multiplexing

Sequencer Runs
- Next Generation systems: 4 to 27 hours per run depending on amplicon size
- Sanger sequencing: 30-40 minutes per 48 or 96 well injection
CDC Supports Newborn Screening Molecular Testing

- Quality Assurance / Proficiency Testing Programs
- Technical Assistance
- Laboratory Training Workshops
  - SCID Training Workshop
  - Newborn Screening Molecular Training Workshop
- Newborn Screening Molecular Resources Website
- Molecular Assessment Program (MAP) site visit program
Determining Pathogenicity of Sequence Variants

- ACMG workgroup consisting of ACMG, AMP and CAP experts determined 5 categories to classify variants:
  - Known pathogenic
  - Likely to be pathogenic
  - Unknown significance
  - Likely to be benign
  - Benign

- Knowledge accruing daily, however the medical impact of most variants is unknown

Richards, S. et al. 2015. Genetics in Medicine 17(5):405-424
Scientific Resources to Classify Variants

- Peer reviewed publications
- Curated Databases
  - General databases (ie ClinVar - NCBI)
  - Disorder specific databases (ie CFTR2 - Cystic fibrosis)
- Predictive programs
  - ie SIFT, PolyPhen-2, Mutation Taster, Condel, PROVEAN, GeneSplicer etc
- ACMG proposed criteria to determine pathogenicity

R. Lee, Ph.D.
Cystic Fibrosis – **CFTR** gene

Pathogenic mutation

ClinVar – “pathogenic”

CFTR2 – “pathogenic”

- **This variant causes CF when combined with another CF-causing variant.** (The other CF-causing variant does not have to be variant F508del. It can be a different variant that also causes CF.)

- **This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.**
  - Patients with this variant will probably need to take oral pancreatic enzyme supplements every day.
  - The oral supplements help the patients’ bodies to absorb the nutrients and vitamins contained in the food they eat.
  - The oral pancreatic supplements will not prevent patients from developing CF.

- There are 64,868 patients with this variant in the CFTR2 database.
Cystic Fibrosis – *CFTR* gene

Conflicting Data

ClinVar – “pathogenic”

CFTR2 – “varying consequences”
Cystic Fibrosis – **CFTR** gene

Variant of Unknown Significance

**ClinVar** – “unknown”

**CFTR2** – “unknown”
Cystic Fibrosis – *CFTR* gene

Benign Variant

ClinVar – “benign”

CFTR2 – “benign”
Krabbe Newborn Screening
Utility of GALC Sequencing

- Recessive genetic disorder that affects the central and peripheral nervous systems
- Onset can vary from first few weeks of life into adulthood
  - Intent of NBS – detection of Early Infantile Krabbe
- Step 1: Identify decreased GALC enzyme activity
- Step 2: Test for variants in the GALC gene using DNA sequencing and deletion analysis
Impact of 2nd Tier Sequencing on Krabbe Screening in New York

NY Annual birth rate: 
~250,000

1st tier
Detection of ↓GALC Activity
(<20% daily mean):
~1225 babies

1st tier reflex
Detection of ↓GALC Activity
(<12% daily mean):
~75 babies

2nd tier
Presence of at least 1 GALC Variant:
~42 babies

Only these babies are sent for clinical diagnostic evaluation

M. Caggana, Sc.D
Inherited recessive chronic disease caused by mutations in the \textit{CFTR} gene - affects the lungs and digestive system

- Step 1: Identify elevated IRT
- Step 2: Test for CF causing variants in \textit{CFTR} gene
  - Screen positive = 1 or 2 CF causing variants
- Step 3: Sequence samples with only 1 CF causing variant

Cystic Fibrosis Newborn Screening Utility of \textit{CFTR} Sequencing
Impact of 3rd Tier Sequencing on Cystic Fibrosis Screening in California

CA Annual birth rate:
~500,000

Babies ≥ 62 ng/mL IRT:
~8,300

Babies with 1 or 2 CFTR variants: ~600

Babies with 2 CFTR variants: ~170

Only these babies are sent for clinical diagnostic evaluation

R. Olney, MD, MPH
Conclusions

- Newborn screening utilizes sequencing for select disorders
- Most programs use Sanger sequencing - research is ongoing into utility of Next Gen sequencing
  - Gene size, throughput, turnaround time
- CDC and APHL supports newborn screening with quality assurance, training and technical support
- Classifying variants involves a lot unknowns
- Sequencing as a 2nd or 3rd tier can enhance specificity of a biochemical test reducing false positives
Thank you!

Newborn Screening

Saving Lives.

Promoting Healthier Babies.

Protecting our Future.

For more information please contact Centers for Disease Control and Prevention
1600 Clifton Road NE, Atlanta, GA 30333
Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov   Web: www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.