Sequencing Applications in Newborn Screening

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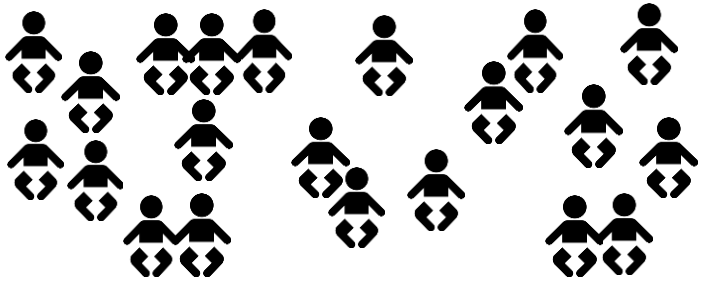
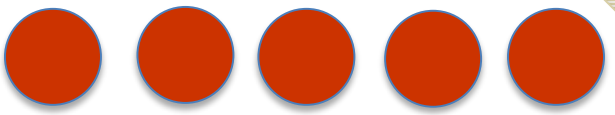


4 million babies a year

NBS specimens



NBS lab testing

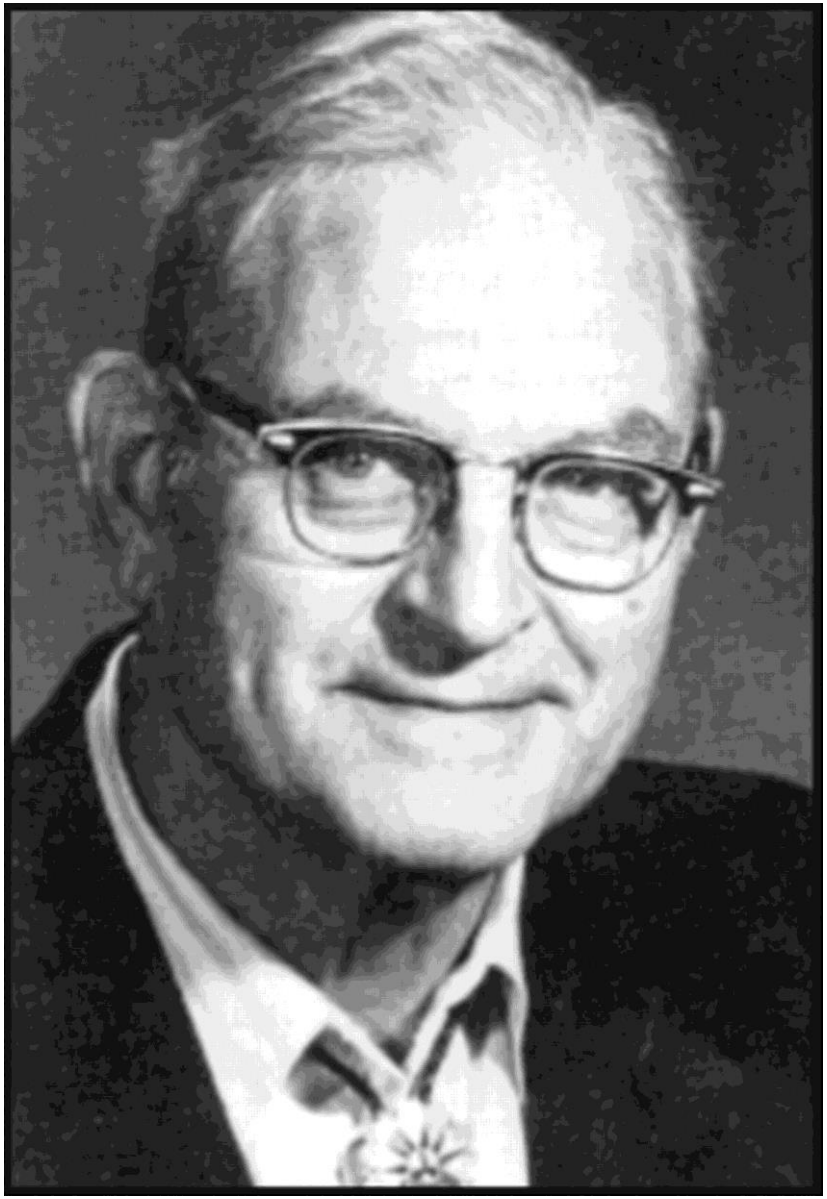
Confirmatory Testing

Dx

Early Rx

PCPs
Specialists

Good Outcome



Robert Guthrie, MD, PhD
(1916-1995)

Advances in technology and methodology have often driven improved screening procedures!

Molecular Testing in WI NBS

- **Targeted variants panel as second tier testing for CF**
  - ✓ 1994 F508del
  - ✓ 2003 *CFTR* 23 variants panel using ASO and Invader
  - ✓ 2017 *CFTR* 276 variants panel using **NGS**
  - ✓ 2020 *CFTR* 345 variants panel using NGS based on CFTR2 update
- **Gene variants as just-in-time information**
  - ✓ 2005 Targeted variants in *GALT*, *BCKDHA* using **tetra-primer ARMS-PCR**
  - ✓ 2005 Targeted variants in *ACADM* and *PCCB* using Restriction Fragment Length Polymorphism (**RFLP**)
  - ✓ 2016 Targeted variants in *HBB* and *RAG1* using **Sanger sequencing**
  - ✓ 2017 *GAA* gene analysis using Sanger sequencing
  - ✓ 2019 *SMN2* copy numbers using droplet digital PCR (**ddPCR)**
- **TREC and *SMN1* as first tier testing for SCID and SMA respectively**
  - ✓ 2008 T-cell excision circles (TREC) using duplex real-time PCR
  - ✓ 2019 TREC / *SMN1* / *RPP30* using **triplex real-time PCR**

Molecular Testing in WI NBS

- **Primary markers**
  - ✓ TREC for SCID
  - ✓ SMN1 zero for SMA
- **Second tier markers**
  - ✓ *CFTR* pathogenic variants for CF after IRT
- **Just-in-time information**
  - ✓ *GALT* mutation panel in galactosemia
  - ✓ *ACADM* mutations in MCADD
  - ✓ *BCKDHA* c.1325 T>A mutation in MSUD and *PCCB* c.1606 A>G in propionic acidemia Both are common variants in Plain population.
  - ✓ *HBB* c.20 A>T and c.19 G>A in sickle cell disease
  - ✓ Whole gene sequence in Pompe
  - ✓ *SMN2* copy numbers in SMA

Gene Segment vs. Whole Gene

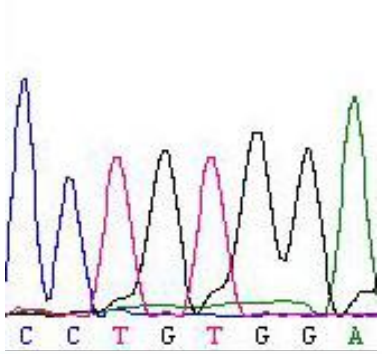
- **Targeted gene variant (s)**
  - Sequence segment (S) containing interested variant (s)
  - Known disease causing variants

- **Whole gene sequencing**
  - Gene coding regions and exon/intron flanking regions
  - *De novo* disease causing variants
  - Variants of unknown significance (VOUS)

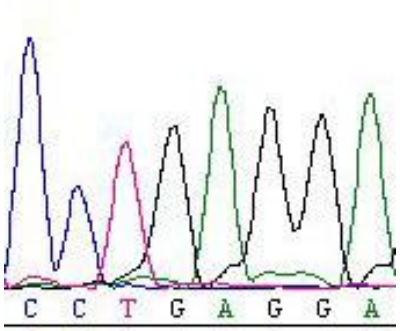
Targeted *HBB* Sequencing

- **Sickle cell disease and hemoglobin SC disease**
- **Screening protocol**
  - First tier: hemoglobin pattern in IEF
  - Second tier: HPLC
- **Apparent "sickle cell trait" in blood transfusion specimens**

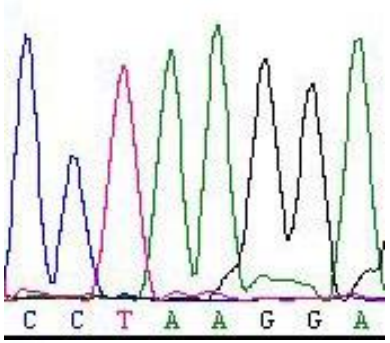
HBB c.20A>T and c.19G>A Detection by Sanger Sequencing



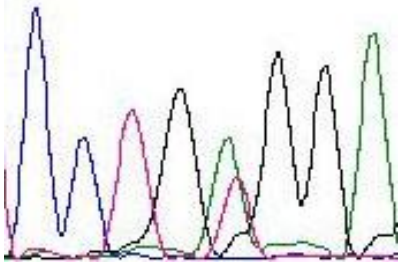
HGB S



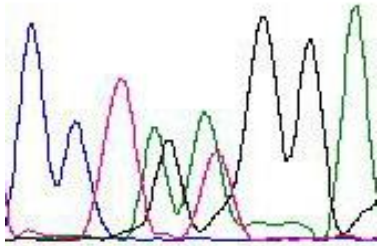
HGB Normal



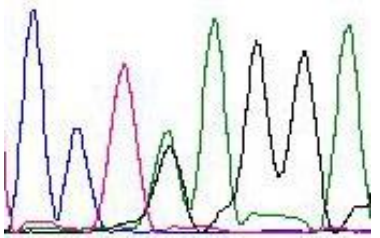
HGB C



HGB S Trait



HGB S/C

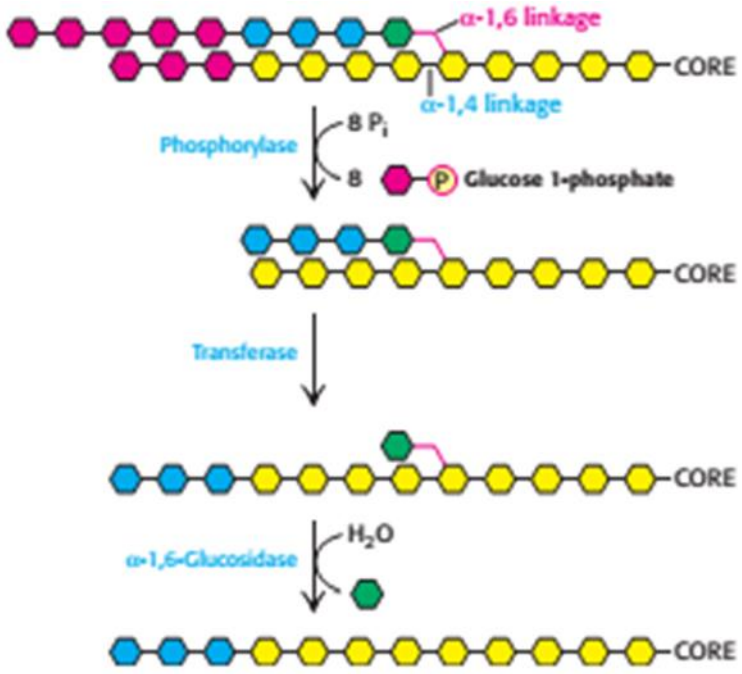


HGB C Trait

Pompe Disease

Etiology

- The *GAA* gene on chromosome 17q25.3 synthesizes acid alfa-Glucosidase (GAA), which is a lysosomal enzyme catalyzing alpha 1,4 and alpha 1,6 linkages of lysosomal glycogen. Mutations in such a gene inherited in an autosomal recessive fashion, lead to GAA deficient or null.



Pathophysiology

- Lysosomal accumulation of glycogen in all tissues, most notably skeletal muscles

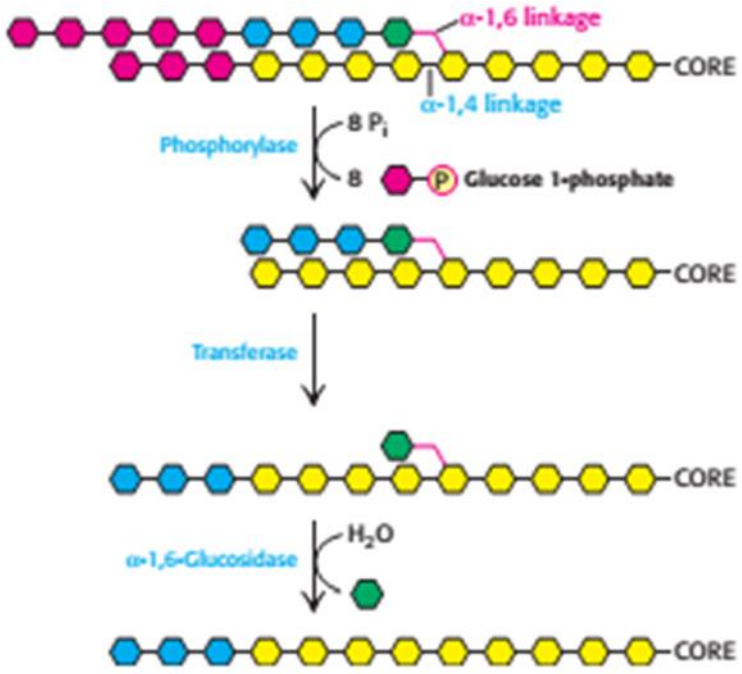
Pompe Disease

Clinical Presentation

- Hypertrophic cardiomyopathy led to heart failure
- Skeletal and respiratory muscle weakness
- Infantile and late-onset presentations

Treatment

- Enzyme replacement therapy
  - ✓ IOPD-prolongs survival, ventilator-free survival and reverses cardiomegaly
  - ✓ LOPD-improved motor capability and stabilized pulmonary function
  - ✓ CRIM status



NBS Testing for Pompe Disease

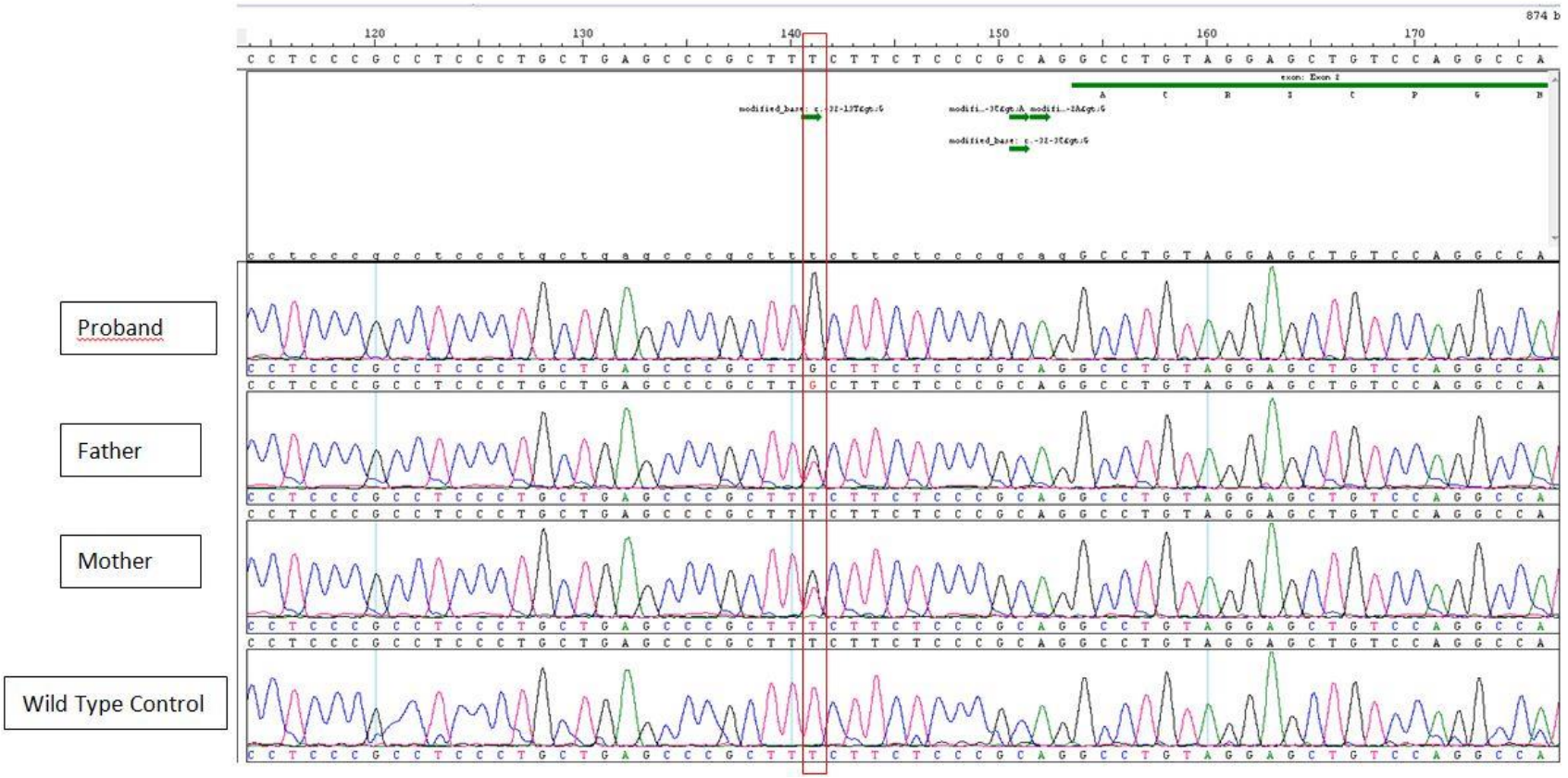
- Fluorometric assay
- Digital microfluidics platform
- Tandem mass spectrometry method
  - Perform enzyme reaction by converting substrate into product
  - Quantitate the product by MSMS as a function of enzymatic activity (nmol/mL/hr)

Pompe NBS Pilot Procedure

- ***GAA* activity**
  - Abnormal: *GAA* is less than 10% of the daily median
  - Possible abnormal: *GAA* is 10-15% of the daily median
  - Inconclusive: multiple low enzyme activities
- **CLIR assessment**
- **Mayo PD2T**
- ***GAA* gene sequencing**

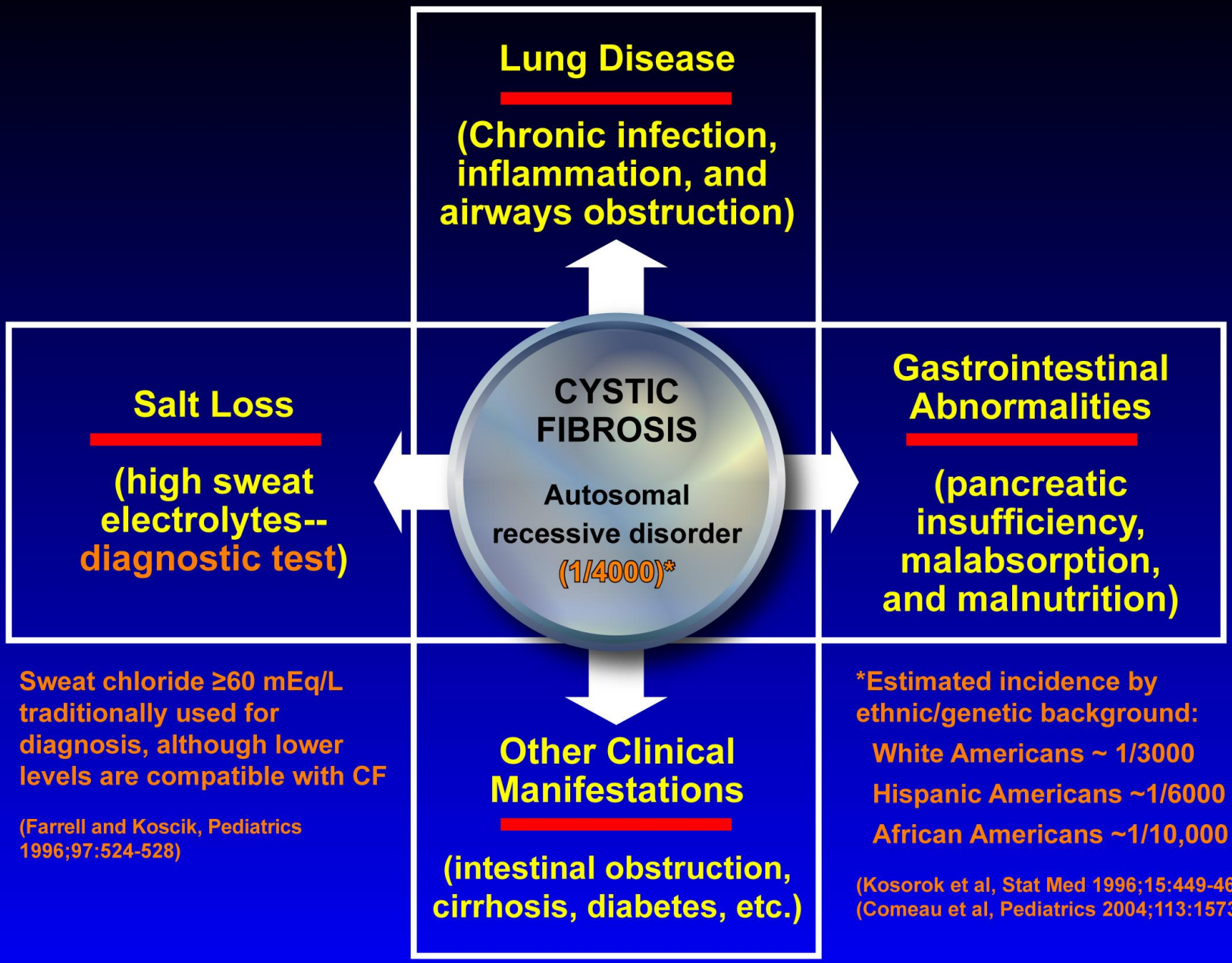
  Further assessment for IOPD, LOPD, and pseudo deficiency

GAA Variants Detection by Sanger Sequencing



Sanger Sequencing vs. NGS

- **Sanger sequencing**
  - One gene segment at a time
  - Choice of method for multiple variants in single individual sample or single variant in multiple individual samples
  - Simple data analysis
- **Next generation sequencing (NGS)**
  - “Unlimited” gene segments
  - Simultaneously assess “unlimited” variants in multiple individual samples
  - Complex data analysis and management



Sweat chloride ≥60 mEq/L traditionally used for diagnosis, although lower levels are compatible with CF

(Farrell and Koscik, Pediatrics 1996;97:524-528)

*Estimated incidence by ethnic/genetic background:

White Americans ~ 1/3000

Hispanic Americans ~1/6000

African Americans ~1/10,000

(Kosorok et al, Stat Med 1996;15:449-462)
(Comeau et al, Pediatrics 2004;113:1573-1581)

Progression of CF NBS Tests

IRT →	IRT/DNA (F508del) →	IRT/DNA (CFTR-23) →	IRT/DNA/DNA* (CFTR > 200)
1979 →	1991 →	2003 →	2012-16

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

CFTR2 Variant List History

	V4 8/2015	V5 8/2016	V6 3/2017	V7 12/2017	V8 8/2018	V9 3/2019	V10 1/2020
Number of Patients	88,664	88,664	88,664	88,664	89,052	89,052	89,052
CF-causing	242	272	281	312	336	346	352
Varying Clinical Consequence	19	19	21	36	35	37	46
Non CF-causing	12	12	12	13	20	21	23
Unknown Significance	3	3	8	13	9	8	11
Total	276	306	322	374	400	412	432

Technical Feasibility Study

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

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

Mei W. Baker, MD[1,2], Anne E. Atkins, MPH[2], Suzanne K. Cordovado, PhD[3], Miyono Hendrix, MS[3], Marie C. Earley, PhD[3] and Philip M. Farrell, MD, PhD[1,4]

Purpose: Many regions have implemented newborn screening (NBS) for cystic fibrosis (CF) using a limited panel of cystic fibrosis transmembrane regulator (*CFTR*) mutations after immunoreactive trypsinogen (IRT) analysis. We sought to assess the feasibility of further improving the screening using next-generation sequencing (NGS) technology.

Methods: An NGS assay was used to detect 162 *CFTR* mutations/variants characterized by the CFTR2 project. We used 67 dried blood spots (DBSs) containing 48 distinct *CFTR* mutations to validate the assay. NGS assay was retrospectively performed on 165 CF screen–positive samples with one *CFTR* mutation.

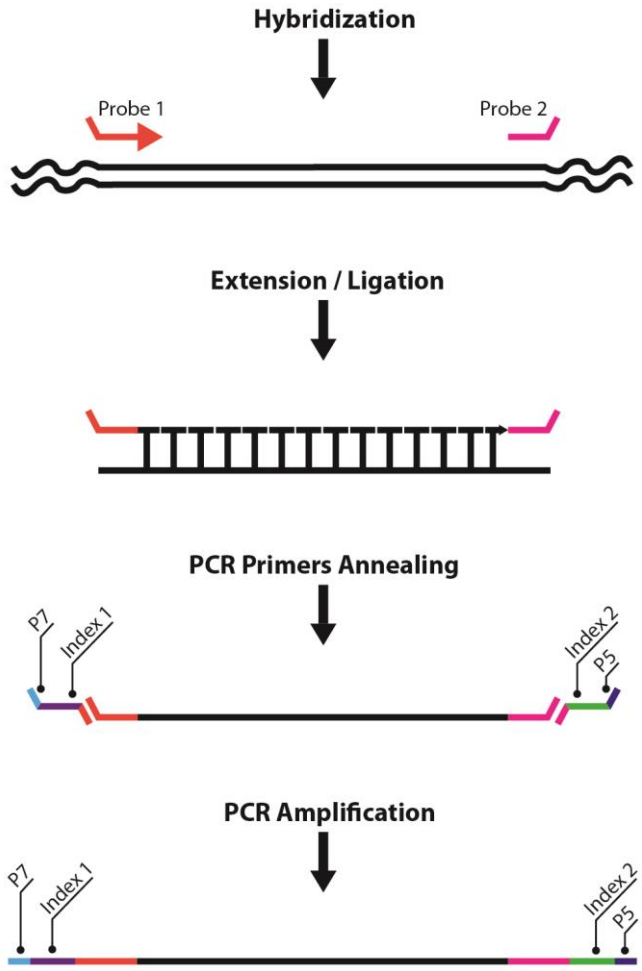
Results: The NGS assay was successfully performed using DNA isolated from DBSs, and it correctly detected all *CFTR* mutations in the validation. Among 165 screen-positive infants with one *CFTR* mutation, no additional disease-causing mutation was identified in 151 samples consistent with normal sweat tests. Five infants had a CF-causing mutation that was not included in this panel, and nine with two CF-causing mutations were identified.

Conclusion: The NGS assay was 100% concordant with traditional methods. Retrospective analysis results indicate an IRT/NGS screening algorithm would enable high sensitivity, better specificity and positive predictive value (PPV). This study lays the foundation for prospective studies and for introducing NGS in NBS laboratories.

Genet Med advance online publication 12 February 2015

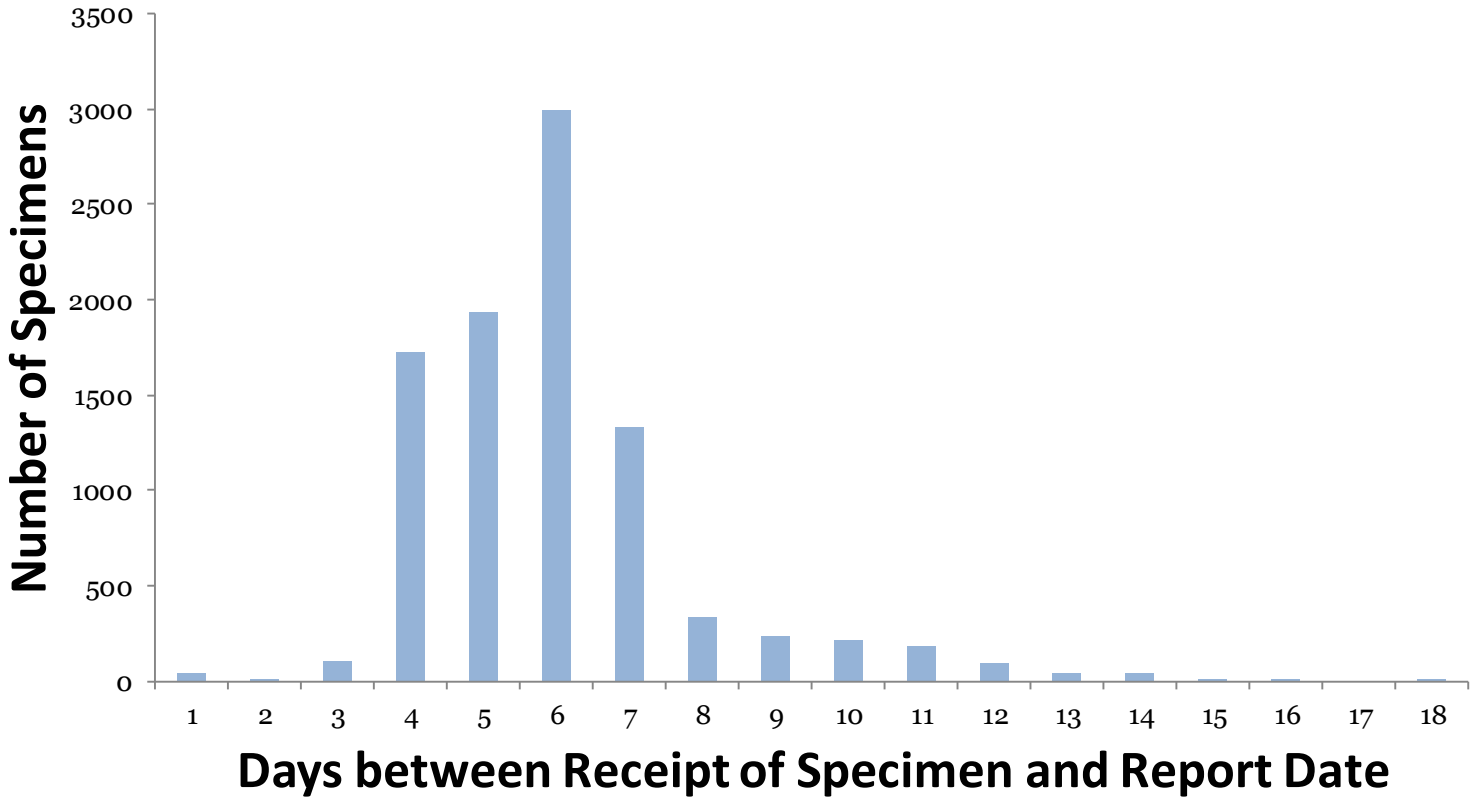
Key Words: cystic fibrosis; cystic fibrosis transmembrane conductance regulator; immunoreactive trypsinogen; newborn screening; next-generation sequencing

NGS *CFTR* Variant 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.

NGS Timeliness in CF NBS

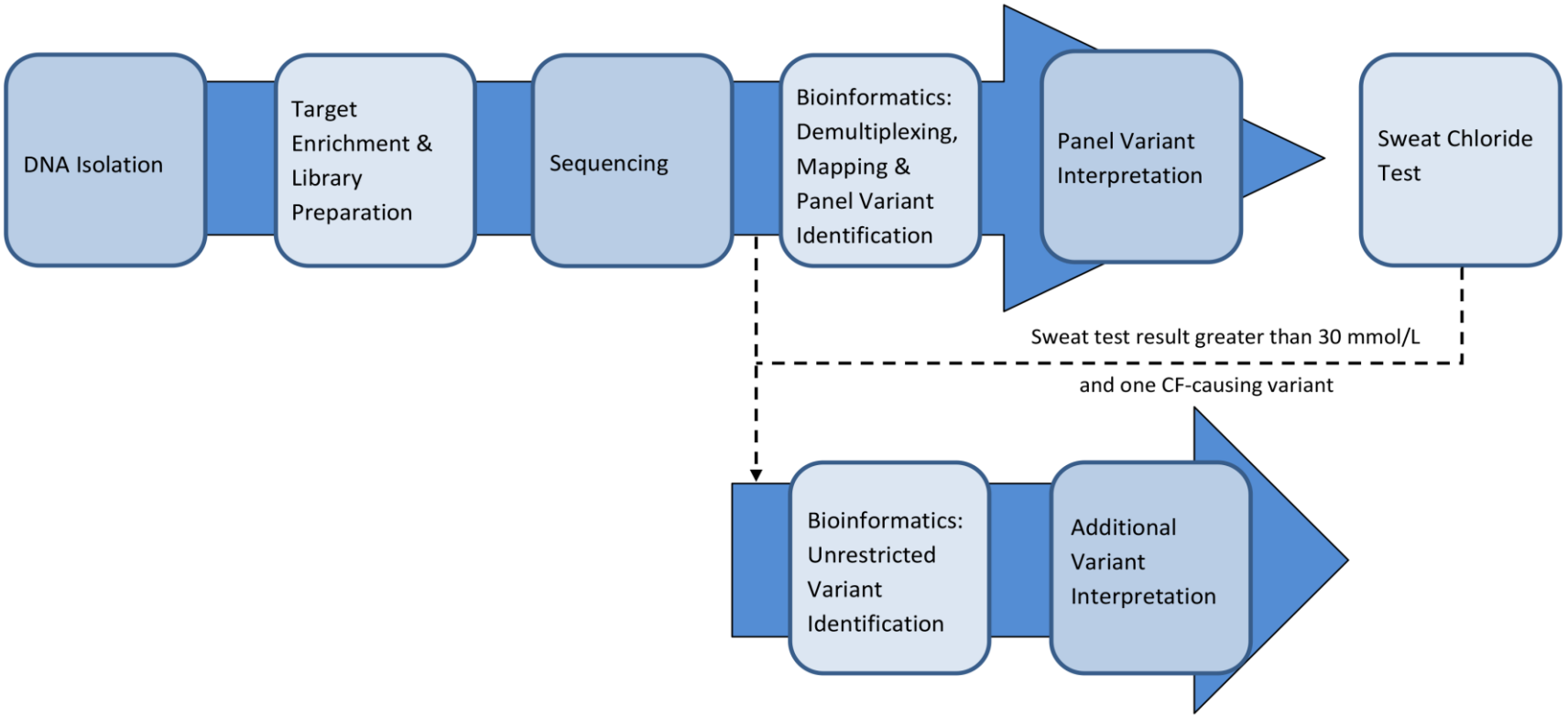


CFTR NGS Assay Performance

April 1, 2016 – March 31, 2019

Category	Number of Runs	Percentage
Successful Run	513	96.1%
Instrument Issues	6	1.1%
Issues Unspecified	11	3.1%
Positive Control Failure	3	0.6%
Power Outage	1	0.2%
Total	534	100%

NGS Integration in NBS for CF



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Newborn Screening for the 21st Century

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

NGS for NBS: Are we there yet?

Not yet, but we are well on the way.

- Clinical utility driven
- Screening performance driven
- Tier approach
- Staged approach

Acknowledgments


Thank You
Asante
Merci
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Kiitos
Welalin
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Matondo
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Vinaka
Dank Je
Kia Ora
Mochchakkeram
Ua Tsaug Rau Koj
Terma Kasih
Matur Nuwun
Chokrane
Nirringrazzjah
Cam on ban
Mamana
Maake

and