2018 APHL™ ANNUAL MEETING

and twelfth government environmental laboratory conference

# Newborn Screening: *It's Complicated!*
# DNA testing in Newborn Screening

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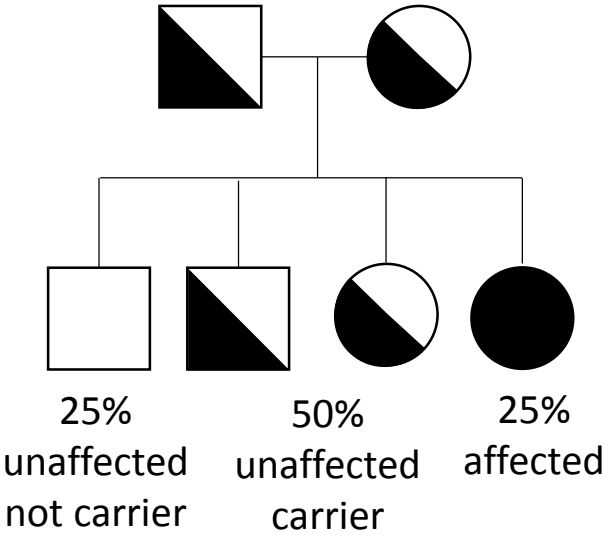
NEW YORK STATE OF OPPORTUNITY. Department of Health | Wadsworth Center

# Why test DNA?

Most conditions screened are genetic…




… is DNA testing necessary?

# Tiered approach

Initial screening biomarker

Immunoreactive trypsinogen (IRT)

Reflex to DNA testing

Mutation panel

# Benefits

Aim for high sensitivity

- ✓ Reduce false positive referrals
- ✓ Rapid molecular dx at birth
- ✓ Complement slow, painful, invasive dx testing
- ✓ Phenotype prediction (some)

- ✓ Triage follow-up
- ✓ Family planning
- ✓ Reduce disparities
- ✓ Molecular therapy

# DNA, tests and conditions

No mutation analysis

Target common mutation(s)

Mutation panels

Gene sequencing

Gene panels

Exome sequencing

Genome sequencing

**Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency**

- Fatty acid oxidation disorder
- 1 in 17,000
- Mendelian, autosomal recessive
- Can't convert certain fats to energy
- Hypoglycemia, lethargy, vomiting, rapid deterioration, seizures, brain damage, coma, sudden death
- Target p.K304E – common mutation
- Dollars/sample, couple hours

C8 → p.K304E

MS/MS target mutation

# DNA, tests and conditions

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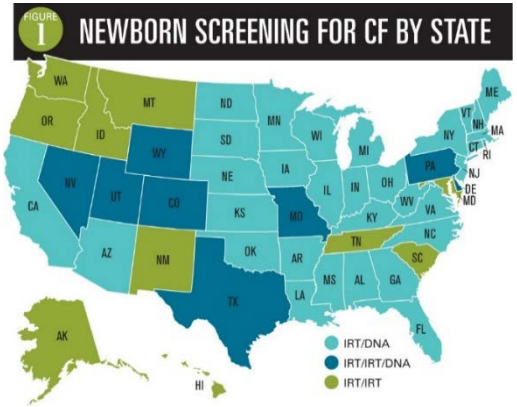
**Cystic Fibrosis (CF)**

- p.F508del – most common, varies by race/ethnicity
- >2,000 *CFTR* gene variants
- Commercial panels – 40–80%
- ~$35/sample, >1 day

IRT → Mutation panel




*Contemporary Pediatrics, 2014*

Abbreviation: CF, cystic fibrosis; DNA, deoxyribonucleic acid; IRT, immunoreactive trypsinogen.
From Cystic Fibrosis Foundation.[16]

# DNA, tests and conditions

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**Pompe disease**

- Lysosomal storage disorder
- 1st tier assay high FPR
- No commercial assays
- No major mutations – diverse
- Pseudodeficiency alleles
- Sequencing (Sanger)
- $ hundreds/sample, >1 day

α-glucosidase activity

↓

Sanger sequence (*GAA*)

# DNA, tests and conditions

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**Severe Combined Immunodeficiency (SCID)**

- Low/absent T-cells (B, NK)
- Lack of adaptive immunity
- Untreated, fatal by 2 yrs
- Spectrum, multiple genes
- Tx varies by genetic defect
- Diagnostics include genetics – iterative, slow, expensive
- Gene panel (NGS)
- $ hundreds/sample, >1 day

2nd tier gene panel (NGS) (research, pilot)

# DNA, tests and conditions

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DNA not needed if # false positives low, positive predictive value high, molecular dx not required

# DNA, tests and conditions

No mutation analysis

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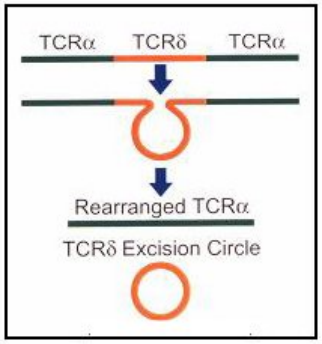
Genome sequencing

**Severe Combined Immunodeficiency (SCID)**

- Low/absent T-cells
- Lack of adaptive immunity
- No biochemical biomarker

*DNA-first molecular* assay (T-cell receptor excision circles; TRECS)




TRECs
↓
Gene panel (NGS)
(research, pilot)

# DNA, tests and conditions

| | NY | TX | WA |
|---|---|---|---|
| **Galactosemia** | 5 common | 4 common | 4 common |
| **Cystic Fibrosis** | Panel (39), NGS | Panel (60) | Panel (planned 2018) |
| **MCADD** | 1 common | 4 common | [No DNA] |
| **VLCADD** | [No DNA] | Seq | [No DNA] |
| **Krabbe Disease** | Seq, 2 del | --- | --- |
| **X-ALD** | Seq | --- | [No DNA] |
| **Pompe Disease** | Seq, 1 del | --- | --- |
| **Hemoglobin-opathies** | --- | PCR, Seq | 4 common |
| **SCID** | TREC (custom) | TREC (custom) | TREC |
| **SMA** | 1 common (pilot) | --- | --- |

--- Condition not screened

# DNA, tests and conditions

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Target common mutation(s)

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Exome sequencing

Genome sequencing

**Spinal Muscular Atrophy (SMA)**

- Neuromuscular disorder
- 1 in 10,000 (most common genetic cause of infant and toddler death)
- Not currently screened, likely soon
  - No biomarker, but primarily caused by single mutation
  - Universal SCID screening (47 states)

Multiplex real-time qPCR: TRECs + SMA mutation

# Pilot studies are essential

Pilot studies ≠ universal, population screening

Retrospective

- Feasibility – infrastructure, logistics
- Evaluate platform, technology, DBS
- Determine reference ranges, cutoffs

Prospective

- Blinded/unblinded
- Data for RUSP assessment
- Human subjects – informed consent (opt in/out)
- Assess population prevalence

Resources – infrastructure, reagents, staff, time

# Logistic Challenges

- Test availability
- Cost
- Infrastructure
- Expertise
- Turnaround time

# Other challenges

- Interpretation difficult
  - Mild, late-onset alleles
  - Variable penetrance or expressivity
  - Variants of uncertain significance (VOUS)
  - Novel variants
  - ‘Patients in waiting’
- Access to genetic counseling
- Provider education
- Screening vs diagnostic testing
- Next generation sequencing


# It's complicated!!!