Newborn Screening for Homocystinuria (HCY) and Congenital Adrenal Hyperplasia (CAH)

Improving the detection of at-risk newborns

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The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC)
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Today's Presentation:

Provide a brief overview of ongoing activities at CDC to improve detection of HCY and CAH

- Not a comprehensive discussion of methodology
- Not a detailed description of ongoing projects

NSMBB's Role in Supporting State NBS Programs

- Method development to detect Newborn Screening conditions
  - New and anticipated additions to the RUSP
  - Improvements to detection conditions already on the RUSP
- Create quality assurance materials and expand performance evaluation programs to respond to the changing needs of the NBS Community
- Provide support for programs to implement screening for anticipated and recently added conditions on the RUSP
- Build capacity and provide technical assistance to troubleshoot current tests and to assist in the implementation of improved screening methods
- Provide education, training – hands on at CDC and on-site

RUSP: recommended uniform screening panel

Homocystinuria: The Basics

- **Classical Homocystinuria is due to a deficiency of Cystathionine β-Synthase**
  - Leads to accumulation of homocysteine
- **Newborn Screening Biomarker is Methionine**
  - Increase in Homocysteine leads to increase in Methionine
  - ↑ Met also seen in liver disease and hyperalimentation
- **Clinical presentation:**
  - Life threatening thromboembolism
  - Seizures, developmental delay, skeletal changes
- **Testing Challenge:**
  - Second tier testing for Homocysteine?
    - Include additional biomarkers – eg Methylmalonic Acid for Cobalamin defects
  - First tier test for homocysteine?
    - Challenging to multiplex with other NBS biomarkers

Background: Public Comments on HCY Screening

Concerns about Disease Detection for Homocystinuria:

- Fifty percent of patients with Classic Homocystinuria are missed
- Methionine is used as screening biomarker (not Homocysteine)
  - ↑ Met also seen in liver disease and hyperalimentation
- Cut off Levels for Methionine are set too high
- Described the benefit of:
  - Reducing current cut-offs for Methionine
  - Second tier test for both Homocysteine and Methylmalonic Acid
  - Developing a first-tier test that includes Homocysteine

Danae Barke, Elizabeth Carter and Margie McGlynn
Homocystinuria Network America
A patient advocacy and patient/family support group

Congenital Adrenal Hyperplasia – The Basics

- **Most cases of CAH are due to 21-Hydroxylase (21OH) Deficiency**
- **Clinical Presentation**
  - Screening identifies classic, severe, forms
    - Salt Wasting (SW) – complete loss of 21OH
    - Simple Virilizing (SV) – partial loss of 21OH
- **Newborn screening biomarker is 17-hydroxy Progesterone (17OHP)**
  - Testing platform is a fluoroimmunoassay (FIA)
- **Reasons for 17OHP FIA false positives**
  - Stress during delivery (↑ 17OHP)
  - Immaturity of adrenal glands (↑ 17OHP)
  - Lack of FIA specificity with other steroid intermediates
- **Current approach: adjust cutoffs and use 2nd tier tests**

There are still false positives and false negatives with current algorithms

Background: Public Comments on CAH Screening

Concerns about Disease Detection for Congenital Adrenal Hyperplasia:

- 17 Hydroxyprogesterone (17OHP) is used as screening biomarker
- Elevations also seen in newborns with prematurity, low birth weight or critical illness
- Endocrine Society: clinical practice guidelines for management of CAH
- Newborn Screening for CAH
  - Improved methods?
  - Standardization?

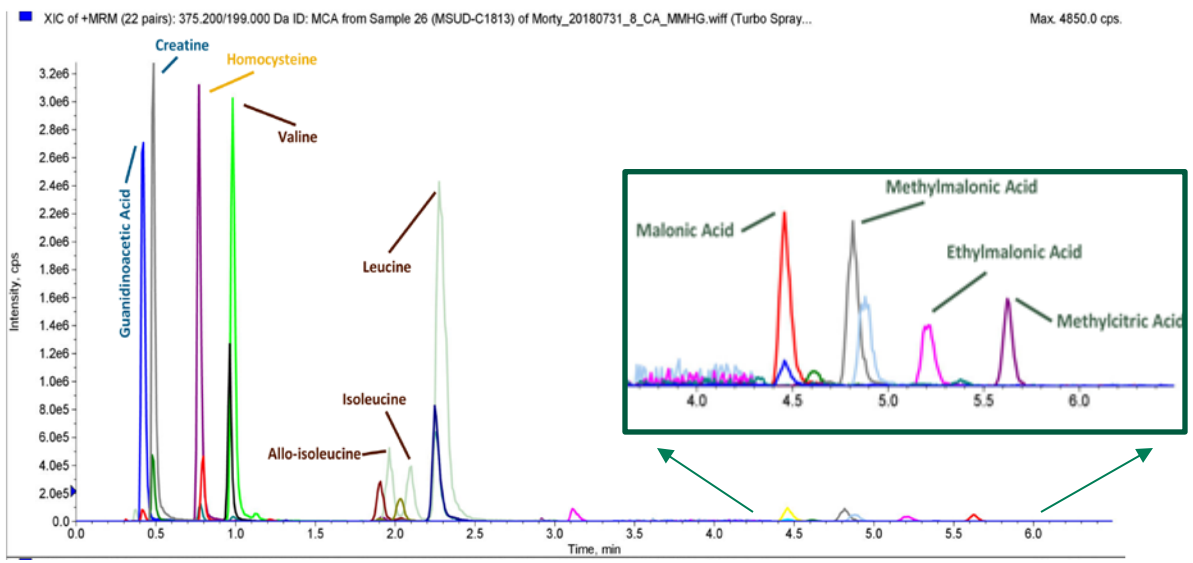
Dr. Emmanuele Delot
Disorders of Sex Development Translational Research Network (DSDTRN)

An NIH-funded national network of clinics and research centers dedicated to improving management of and service to patients with disorders of sex development

Biochemical Approaches to Enhance Detection of HCY and CAH in Newborns

Overview of 4 Methods in Varying Stages of Development

Method #1



GAMT Deficiency Analytes: Blue
Homocystinuria Analyte: Yellow
MSUD Analytes: Maroon
MMA/PA Analytes: Green

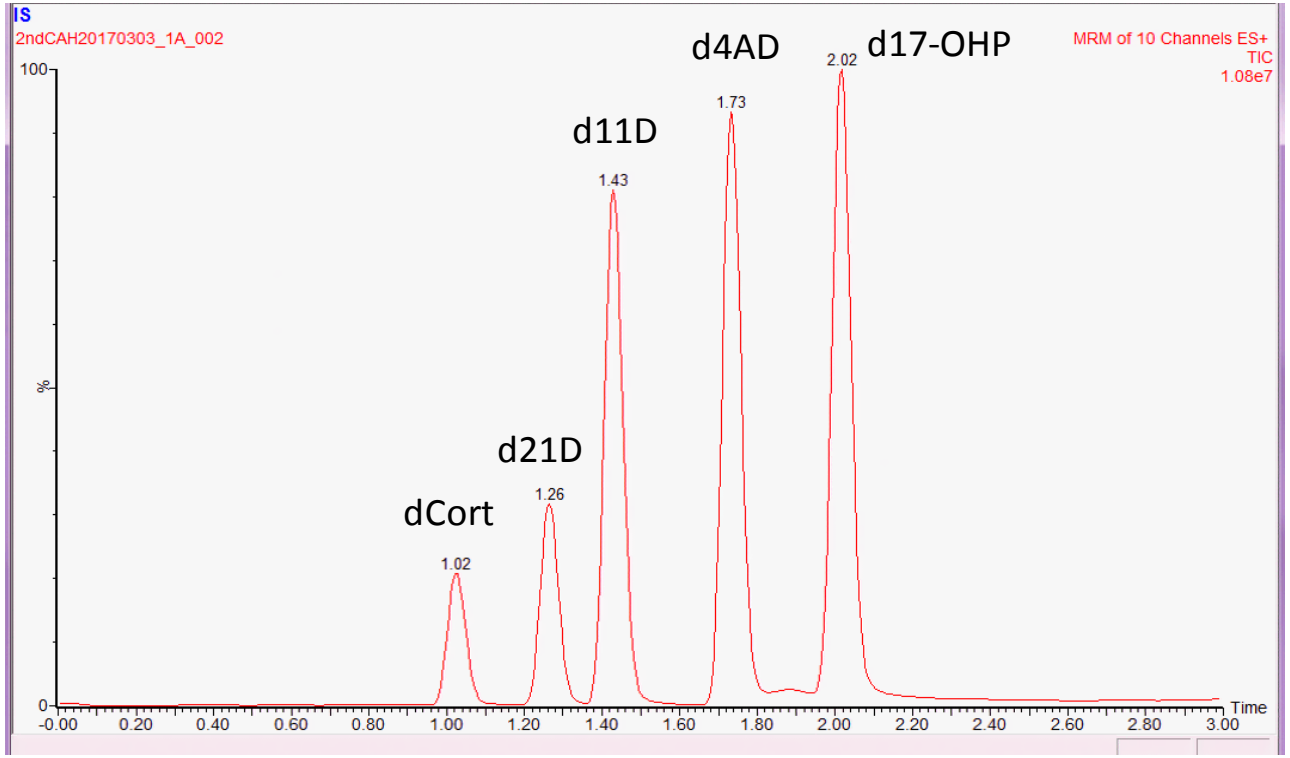
2nd-tier screening:

HCY, MMA, PROP, HCY, GAMT, MSUD

- Reversed phase liquid chromatography of several amino acids and organic acids
- Derivatization to form butyl esters
  - Like existing amino acid/acylcarnitine assay
- Other conditions:
  - Separation: C18 column,
  - Gradient elution, Water:Acetonitrile:Formic acid

Currently being taught to States during Annual MSMS Course

Method #2



17-OHP: 17 Hydroxyprogesterone
4AD: Androstenedione
11D: 11-Deoxycortisol
21D: 21-Deoxycortisol
Cort: Cortisol

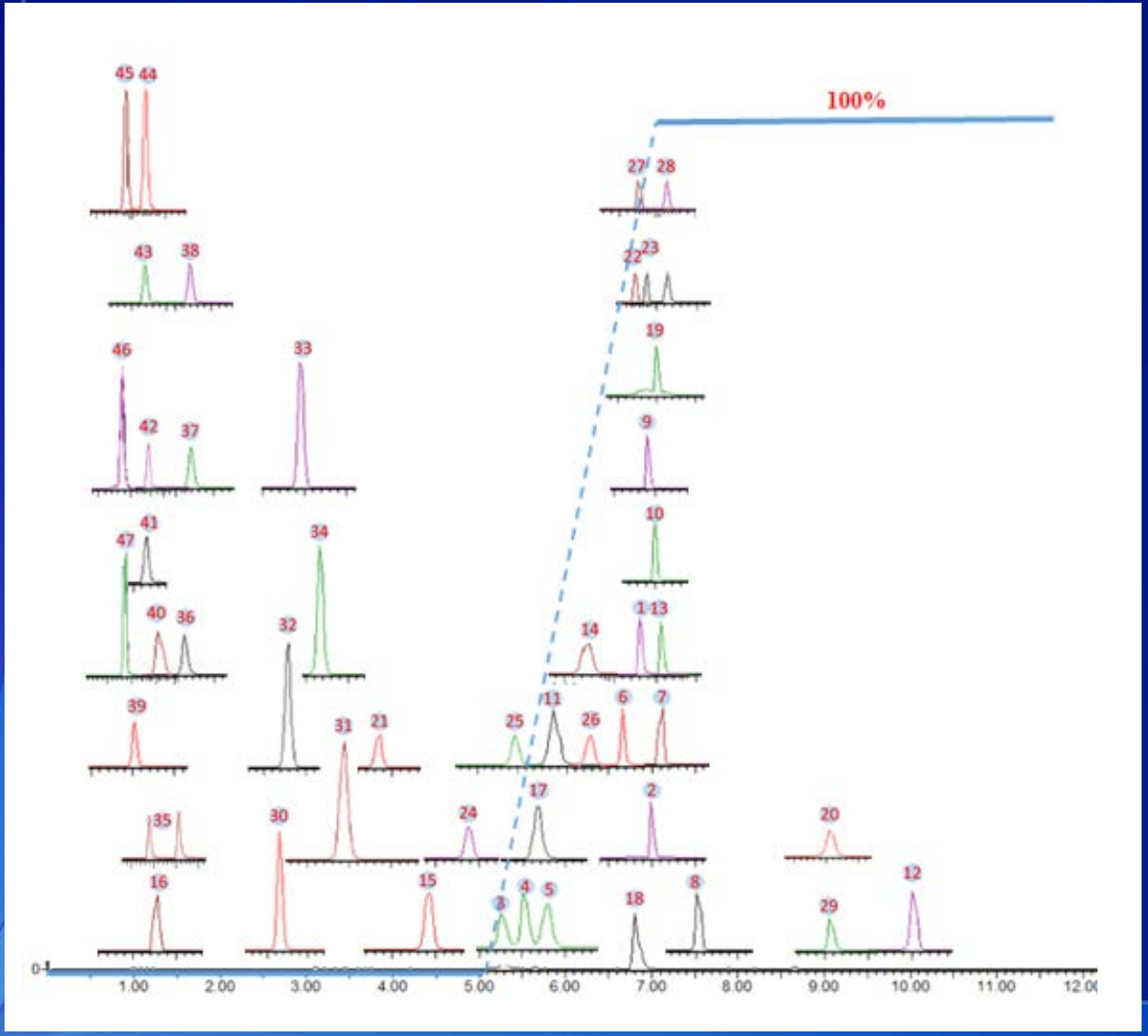
2nd-tier screening:

CAH – Steroid Panel

- **Reversed phase liquid chromatography of steroids**
- **Other conditions:**
  - **Separation: C18 column,**
  - **Gradient elution, Water:Methanol:Formic acid**
- **Only Standards are shown to demonstrate separation**

Will be taught to States during Annual MSMS Course in 2020

Method #3



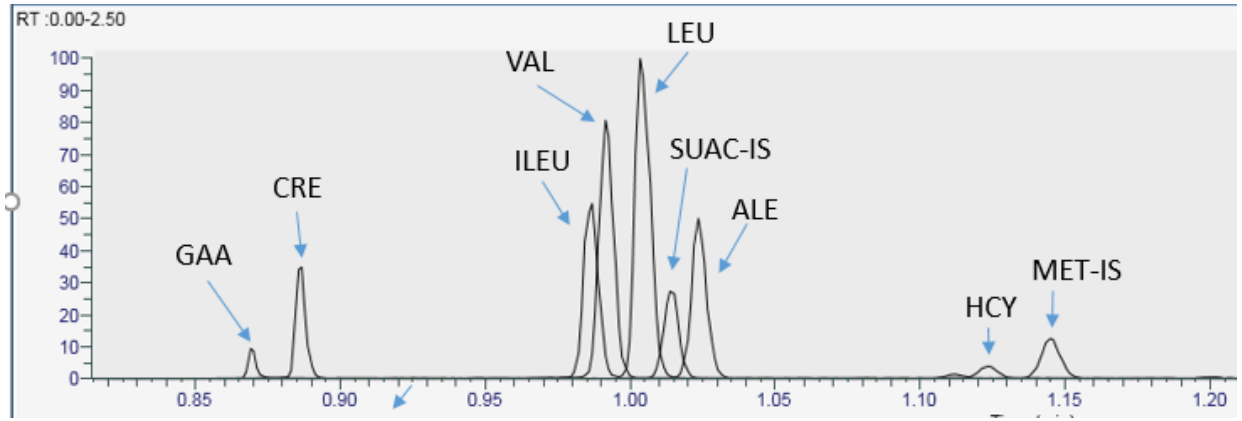
2nd-tier screening:

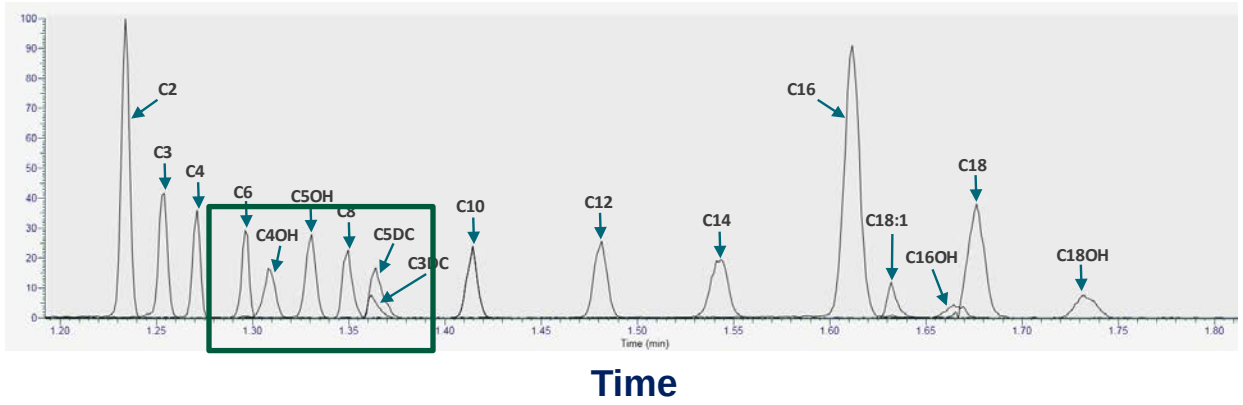
Universal NBS Panel

- **Second-tier Biomarkers to detect HCY, MMA, PROP, HCY, GAMT, MSUD, CAH, X-ALD, GA-I, Pompe**
- **Conditions:**
  - HILIC-MS/MS of amino acids, acylcarntines, LPCs, organic acids, steroids
    - HILIC: Hydrophilic Interaction Liquid Chromatography
  - Gradient elution, Water:Acetonitrile:Additives

Still Under Development

Method #4





1st and 2nd tier markers *combined screening*:

- **Single platform to detect simultaneously detect primary and secondary disease biomarkers**
- **Ultra-high throughput on-chip CE-MS**
  - CE: capillary elecrophoresis
- **Conditions:**
  - Separation: On-Chip Capillary Electrophoresis
  - Buffer: Water:Acetonitrile:Additives

Features
Separates Leucine, isoleucine and Allo-Isoleucine
Separates C3DC and C4OH
Includes GAMT biomarkers

Still Under Development

Training Laboratory Personnel

- **At CDC Campus**
  - APHL/CDC co-sponsored training “Newborn Screening by Tandem Mass Spectrometry: A Hands-On Course in Understanding Laboratory Issues and Interpreting Test Results”
  - 10-12 public health lab personnel per year
    - Classroom sessions on second-tier screening
    - Key biomarkers, biochemical pathways, result interpretation
    - Hands-on laboratory part on second-tier screening
    - Sample preparation, LC-MS/MS analysis and result review

- **As needed in public health labs:**
  - 1-2 CDC employees spend 3 days training NBS staff on mass spectrometry based second-tier screening approaches

Molecular Approach to Enhance Detection of CAH in Newborns

Brief description of a 3 year study

Transient 17OHP Levels Unrelated to CAH Creates Both False Positive and False Negative NBS Results

- **External factors affect 17OHP at birth independent of 21OH activity**
  - False Positive ex. Birth stress and infant immaturity (above infant 17OHP)
  - False Negative: maternal steroid treatment or high circulating maternal cortisol (below infant 17OHP)

- **CHALLENGE: Need for an alternative NBS test not influenced by:**
  - Timing of sample collection
  - Prematurity or birth stress
  - Cross reactivity with other steroids

How can we increase sensitivity by lowering 17OHP cutoffs to eliminate false negatives and use a 2nd-tier CYP21A2 molecular assay to maintain screening specificity?

CAH Molecular Second Tier Screening Study

3-Year March of Dimes Grant

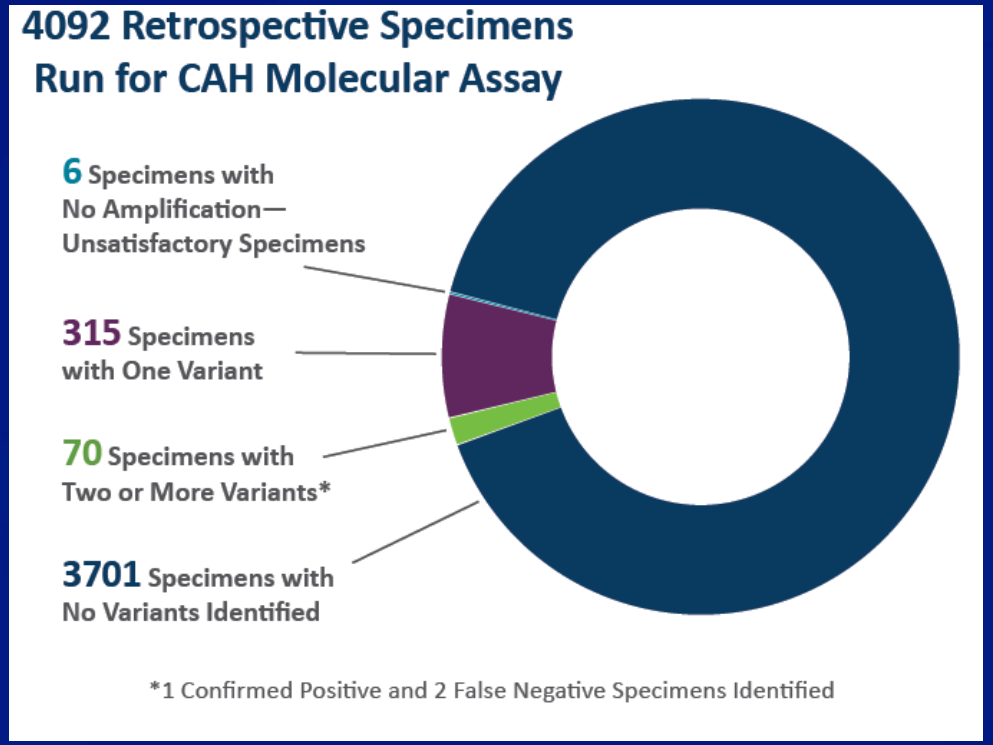
- **Grant Title: “Can molecular testing improve newborn screening performance and outcomes for CAH?”**
  - Grant Co-Investigators
    - University of Minnesota – Principle Investigator
    - Minnesota Department of Health
    - CDC’s Newborn Screening and Molecular Biology Branch
  - Minnesota CAH Clinical Research Consortium (Patient Sources)
    - University of Minnesota
    - Minnesota Children’s Hospital
    - Mayo Clinic

CAH Molecular Second Tier Screening Study

- **Define a Minnesota population CYP21A2 gene variant panel – UMN & CDC**
  - Used family samples
  - Identified a total of 22 CYP21A2 pathogenic variants in addition to the 30kb deletion alleles
- **Develop high-throughput molecular assay for NBS laboratory – CDC**
  - Multiplex Allele-Specific Primer Extension (ASPE) with Luminex xTAG Technology
- **Pilot test to evaluate molecular method assay – MDH and CDC**

Assay Transferred to Minnesota Department of Health: One-Year Molecular CAH Retrospective Study

- **72,000 specimens screened**
- **Identified known true CAH positive**
- **Identified 2 CAH babies missed by current screening algorithm**
  - One missed by primary assay cutoff
  - One missed by 2nd tier assay
- **CDC confirmed MN results by DNA sequencing**
- **Correctly identified all deletions and >0.999 of ASPE genotypes**
  - Probes redesigned for 100%
  - Low incidence of severe alleles not on panel



ASPE: Allele-Specific Primer Extension

CAH Molecular Future Directions

- **Novel State/Federal/Academic collaboration as a model for future NBS molecular test development**
  - Establish a comprehensive *CYP21A2* panel for diverse state populations
  - Open-source method allows creation of customized panels
- **Molecular CAH results will require in-depth reporting infrastructure development**
  - Samples with only a single variant – potential for high false positive rate
  - Common to have multiple *CYP21A2* variants on same chromosome
- **DBS phasing assay to eliminate need for family testing**
  - Determine if all variants on the same or separate chromosomes
  - Define common *CYP21A2* haplotypes for assay interpretation

Acknowledgments – CAH Study

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Thank you for your attention!

Newborn Screening

***Saving* Lives.**

***Promoting* Healthier Babies.**

***Protecting* our Future.**

For more information please contact Centers for Disease Control and Prevention

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