GBSC 724 Advanced Special Topics in Metabolomics

Population Scale Metabolomics: Newborn Screening

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Lecture Overview

- Introduction and historical perspective
- Disorders
- Methods
- Logistics, ethical issues, and future considerations

Prologue: the Impact of Newborn Screening

- JS was born in1955 with phenylketonuria (PKU). Undiagnosed, he developed severe intellectual disability and was institutionalized at the age of 20.
- JD was born in1965 with PKU. NBS was now available and led to a diagnosis at 2 weeks of age. He was placed on a special diet and grew to be normal adult.

- ES was born in a state without medium chain acyl-CoA dehydrogenase (MCAD) deficiency screening in 1999. Undiagnosed, she died in her sleep at 15 months of age.
- RD was born on the same day, but 20 miles away, just across the border in a state where MCAD screening was offered. She was placed on dietary therapy and grew to be a normal adult.

Newborn Screening: One of the Ten Great Public Health Achievements Worldwide, 2001–2010

"Improvements in technology and endorsement of a uniform newborn-screening panel of diseases have led to earlier life-saving treatment and intervention for at least 4000 additional newborns each year with selected genetic and endocrine disorders."

Morbidity & Mortality Weekly Report. 2011; 60(24):814-818

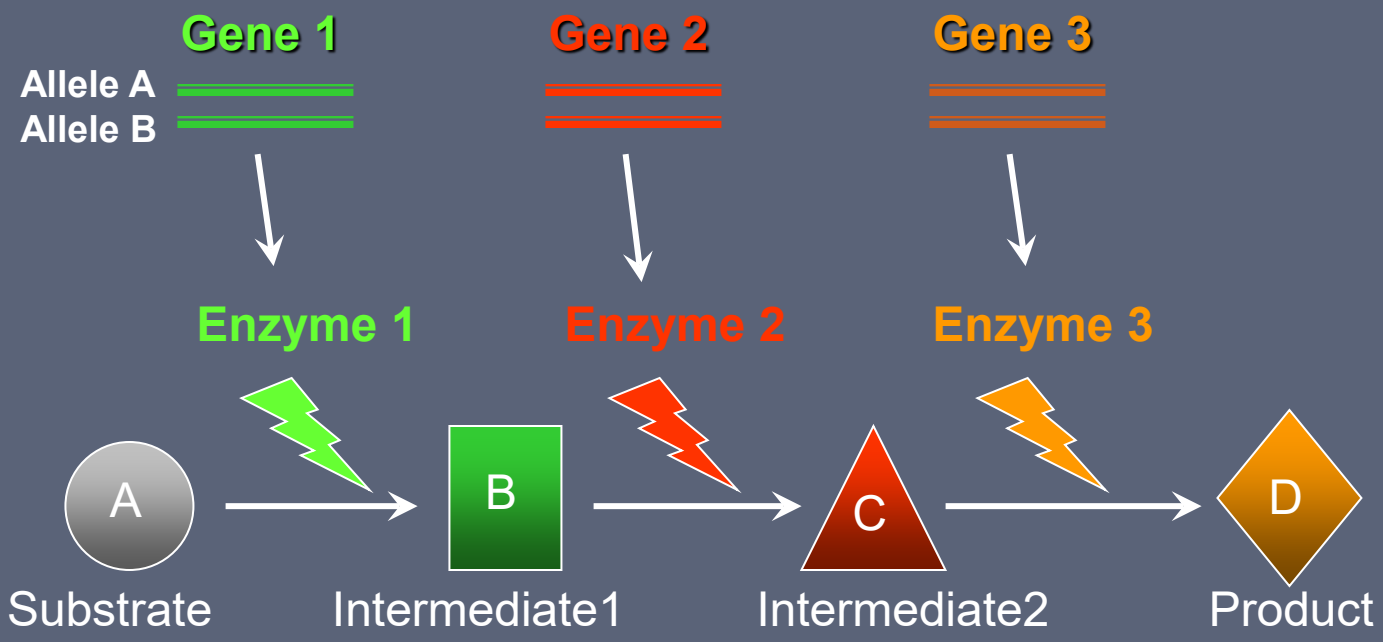
What is Newborn Screening (NBS)?

Approximately 1 in 300 newborns has a condition detectable by modern NBS

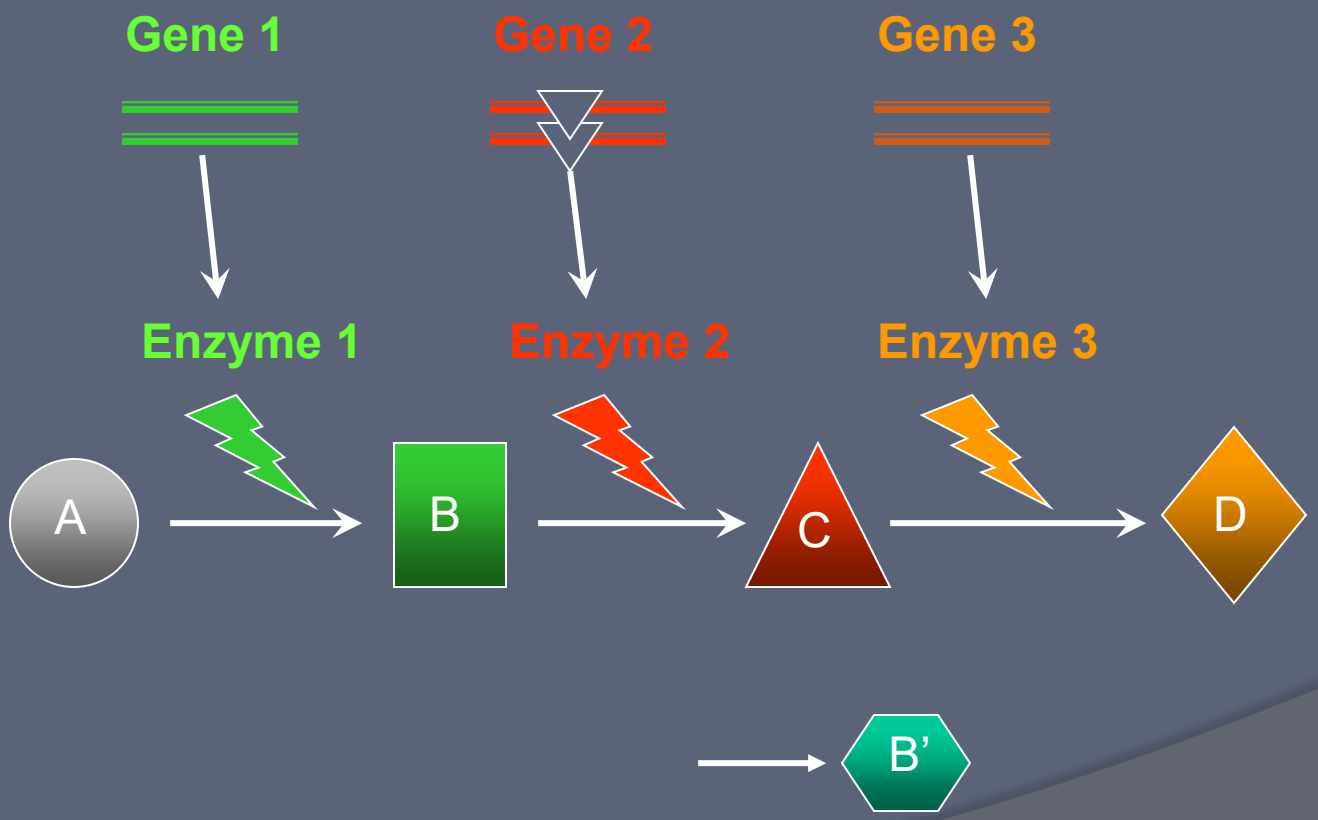
- Population scale screening of all newborns* for the presence of *treatable* conditions that are not otherwise evident at birth
  - screening vs. diagnostic testing
- State – specific programs (no federal mandate) with significant variability
  - disorders detected
  - follow-up procedures

*USA: 4 million births/year

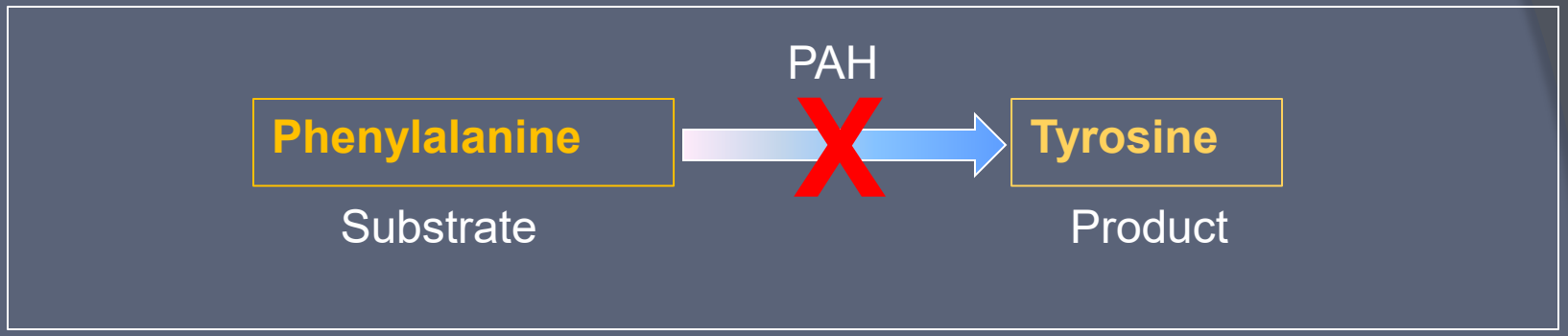
Metabolic Pathways: Sequential Enzyme-catalyzed Reactions



Inherited Metabolic Disorders: Recessive Metabolic Enzyme Dysfunction



The Origins of NBS: Phenylketonuria (PKU)



- Etiology: impaired phenylalanine metabolism, with resulting CNS toxicity
- Treatment: reduction of dietary phenylalanine, but requires early detection
  - Development of a phenylalanine-free formula (Lofenalac)
- Problem: Need a simple test to detect PKU soon after birth, before symptoms arise

Robert Guthrie Pioneered the First NBS Test for PKU in 1961

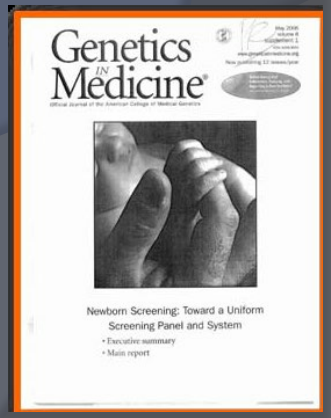
- Filter paper containing blood from newborns applied to a seeded agar plate
- Bacteria only grow in the presence of phenylalanine
  - Large colonies = PKU
- Paradigm: one test for one disorder

A Brief History of Newborn Screening: the Early Years

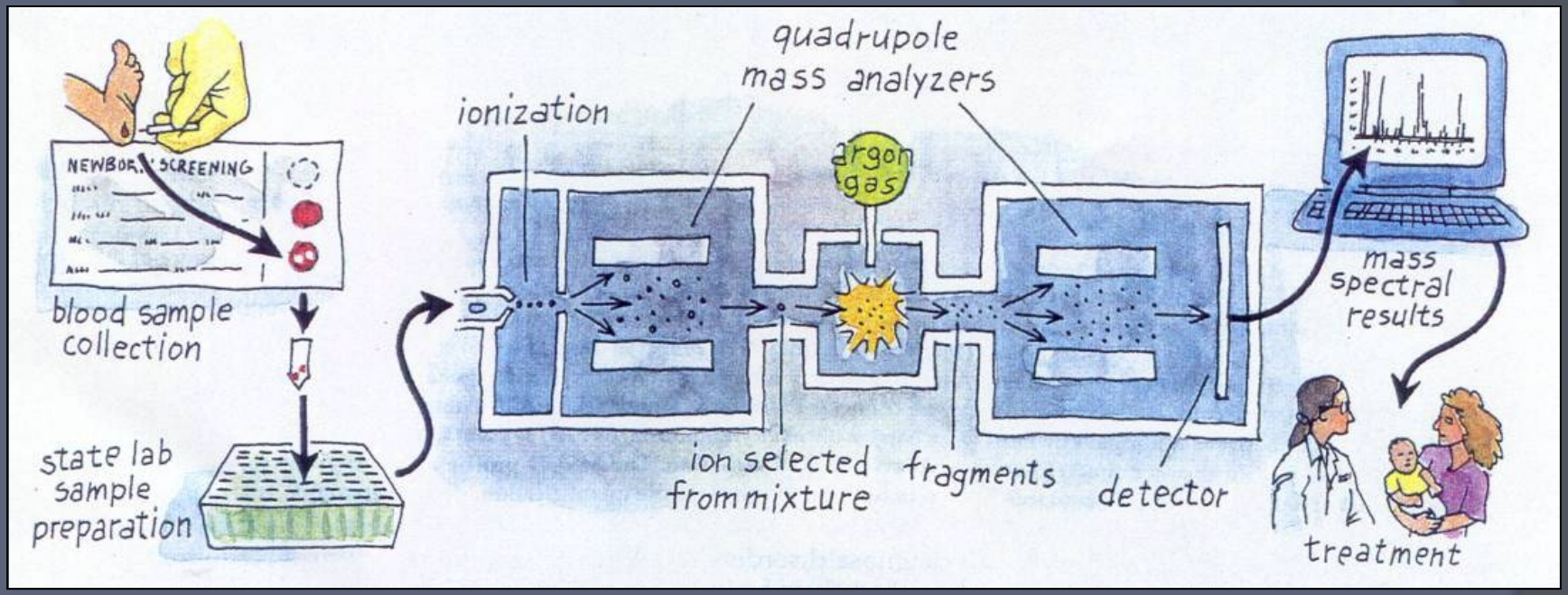
- 1961: Robert Guthrie develops screening test for PKU
- 1962: Massachusetts pilots state-wide PKU screening
- 1965: Over 50% of states have mandated PKU screening
- 1968: WHO publishes *Principles and Practices of Screening for Disease*
  - Wilson-Jungner principles (early screening criteria)
- 1970s - 1990s: most states screen for ~6 conditions

A Brief History of Newborn Screening: the Era of Mass Spectrometry

- 1990s – early 2000s: Development and implementation of MSMS for newborn screening
  - New paradigm: one test for multiple disorders
- 2002: Maternal and Child Health Bureau commissions ACMG to recommend a uniform panel of conditions for NBS
  - 2005: ACMG ENS report identifies 29 core conditions and 25 secondary conditions (designated by HHS as the national standard for NBS – but not federally mandated)
- 2009: All states screen for at least 29 disorders; approximately 20 states screen for 40+ disorders



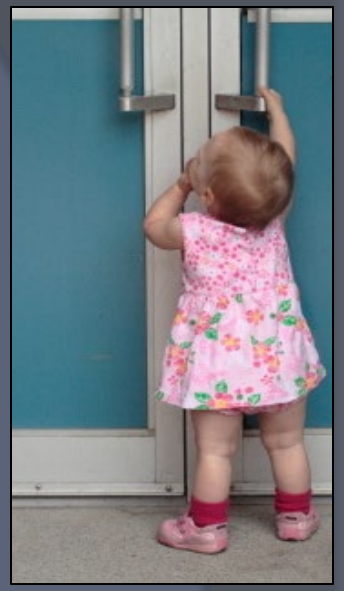
Modern Newborn Screening via Tandem Mass Spectrometry



Blood sample collected 24 – 48 hrs after birth (follow-up screen at 2 – 4 weeks in some states)

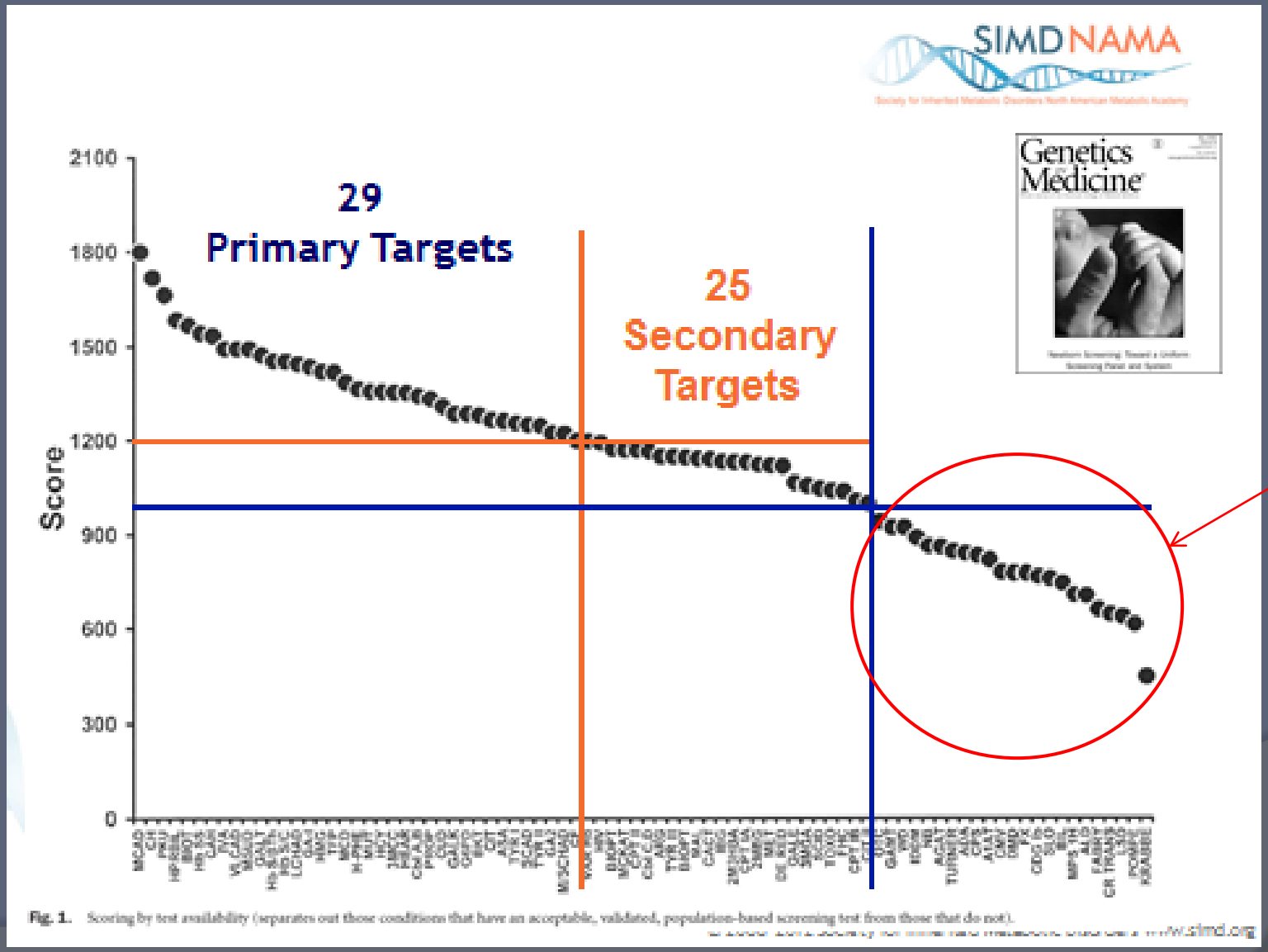
- Analytical time: 5 minutes
- Metabolites detected: >20
- Conditions screened: >50

Criteria for Inclusion in the ACMG Core Screening Panel (2006)



- An effective treatment is available
- Demonstrated benefits of early detection and treatment (clinical utility)
- The condition does not usually produce symptoms within 24 – 48 hrs after birth
- A sensitive, specific, and cost-effective test is available that can detect the condition within this time frame
- See http://mchb.hrsa.gov/screening/ for more about the ENS task force

2005 ACMG Panel Scores



LSDs now being added to many NBS panels

Screened Disorders in the United States

- Currently, 35 core conditions are on the Recommended Uniform Screening Panel (RUSP)
  - 20 classified as metabolic disorders (eg, PKU)
  - 2 endocrine disorders (eg, CAH)
  - 3 hemoglobin disorders (eg, sickle cell anemia)
  - 10 other conditions (eg, hearing loss, cystic fibrosis)
- Also 26 secondary conditions (may lack an effective therapy or have an unclear natural hx) that can be detected when screening for core disorders
  - 22 metabolic
  - 1 hemoglobinopathy
  - 3 other

National Newborn Screening & Global Resource Center (NNSGRC)

HRSA Recommended Uniform Screening Panel (RUSP) Core Conditions 2022

Recommended Uniform Screening Panel
Core Conditions
(As of August 2022)

X: Condition is in this category --: Condition is not in this category

Core Condition	Metabolic Disorder - Organic acid condition	Metabolic Disorder - Fatty acid oxidation disorder	Metabolic Disorder - Amino acid disorder	Endocrine Disorder	Hemoglobin Disorder	Other Disorder
Propionic Acidemia	X	--	--	--	--	--
Methylmalonic Acidemia (methylmalonyl-CoA mutase)	X	--	--	--	--	--
Methylmalonic Acidemia (Cobalamin disorders)	X	--	--	--	--	--
Isovaleric Acidemia	X	--	--	--	--	--
3-Methylcrotonyl-CoA Carboxylase Deficiency	X	--	--	--	--	--
3-Hydroxy-3-Methylglutaric Aciduria	X	--	--	--	--	--
Holocarboxylase Synthase Deficiency	X	--	--	--	--	--
ß-Ketothiolase Deficiency	X	--	--	--	--	--
Glutaric Acidemia Type I	X	--	--	--	--	--
Carnitine Uptake Defect/Carnitine Transport Defect	--	X	--	--	--	--
Medium-chain Acyl-CoA Dehydrogenase Deficiency	--	X	--	--	--	--
Very Long-chain Acyl-CoA Dehydrogenase Deficiency	--	X	--	--	--	--
Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency	--	X	--	--	--	--
Trifunctional Protein Deficiency	--	X	--	--	--	--
Argininosuccinic Aciduria	--	--	X	--	--	--
Citrullinemia, Type I	--	--	X	--	--	--
Maple Syrup Urine Disease	--	--	X	--	--	--
Homocystinuria	--	--	X	--	--	--
Classic Phenylketonuria	--	--	X	--	--	--
Tyrosinemia, Type I	--	--	X	--	--	--
Primary Congenital Hypothyroidism	--	--	--	X	--	--
Congenital adrenal hyperplasia	--	--	--	X	--	--
S,S Disease (Sickle Cell Anemia)	--	--	--	--	X	--
S, βeta-Thalassemia	--	--	--	--	X	--
S,C Disease	--	--	--	--	X	--
Biotinidase Deficiency	--	--	--	--	--	X
Critical Congenital Heart Disease	--	--	--	--	--	X
Cystic Fibrosis	--	--	--	--	--	X
Classic Galactosemia	--	--	--	--	--	X
Glycogen Storage Disease Type II (Pompe)	--	--	--	--	--	X
Hearing Loss	--	--	--	--	--	X

Core Condition - continued	Metabolic Disorder - Organic acid condition	Metabolic Disorder - Fatty acid oxidation disorder	Metabolic Disorder - Amino acid disorder	Endocrine Disorder	Hemoglobin Disorder	Other Disorder
Severe Combined Immunodeficiencies	--	--	--	--	--	X
Mucopolysaccharidosis Type I	--	--	--	--	--	X
X-linked Adrenoleukodystrophy	--	--	--	--	--	X
Spinal Muscular Atrophy due to homozygous deletion of exon 7 in SMN1	--	--	--	--	--	X
Mucopolysaccharidosis Type II	--	--	--	--	--	X

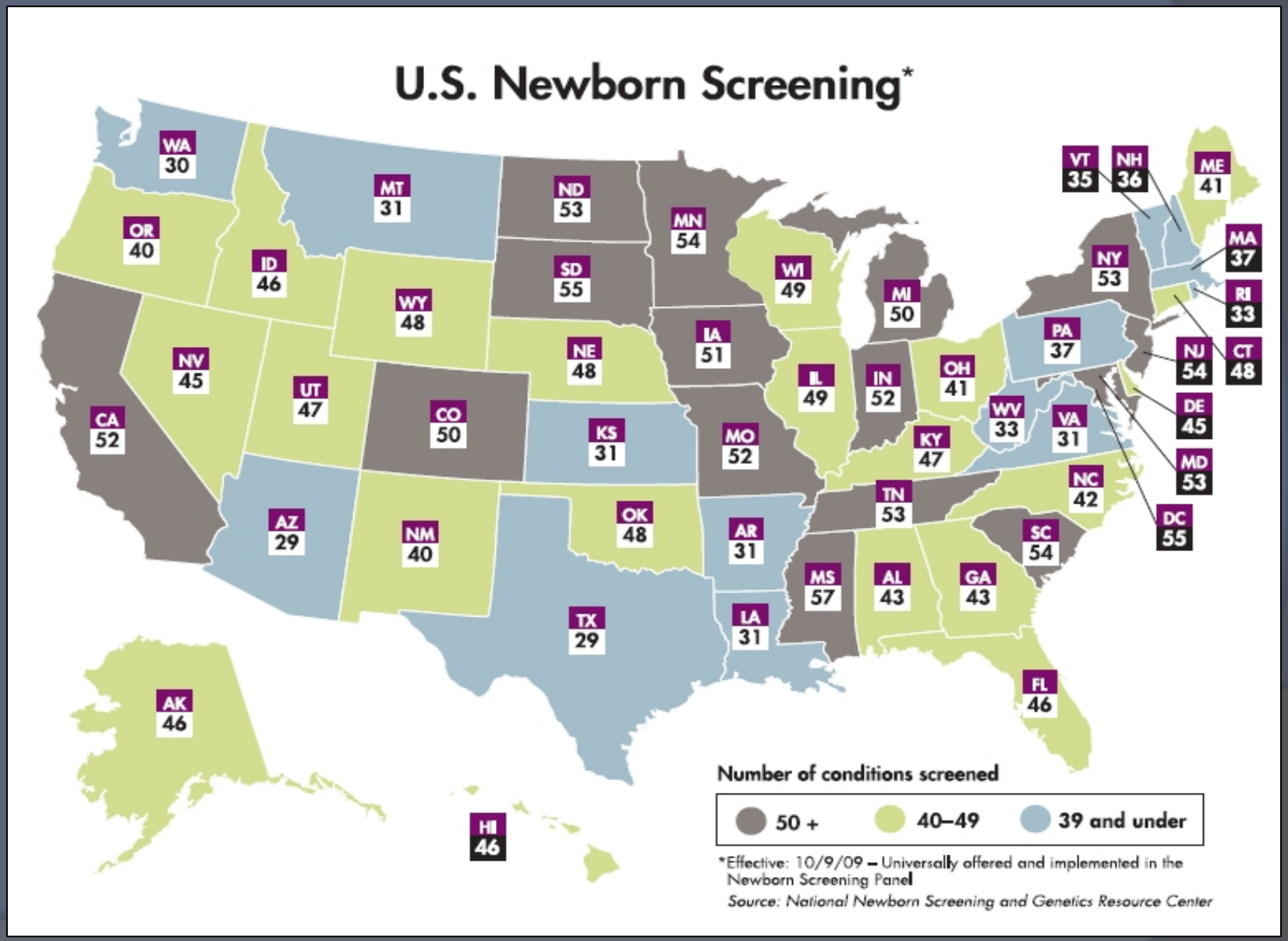
2022 RUSP Secondary Conditions

Recommended Uniform Screening Panel[1]
SECONDARY[2] CONDITIONS[3]
(As of August 2020)

Secondary Condition	Metabolic Disorder - Organic acid condition	Metabolic Disorder - Fatty acid oxidation disorder	Metabolic Disorder - Amino acid disorder	Endocrine Disorder	Hemoglobin Disorder	Other Disorder
Methylmalonic acidemia with homocystinuria	X	--	--	--	--	--
Malonic acidemia	X	--	--	--	--	--
Isobutyrylglycinuria	X	--	--	--	--	--
2-Methylbutyrylglycinuria	X	--	--	--	--	--
3-Methylglutaconic aciduria	X	--	--	--	--	--
2-Methyl-3-hydroxybutyric aciduria	X	--	--	--	--	--
Short-chain acyl-CoA dehydrogenase deficiency	--	X	--	--	--	--
Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	--	X	--	--	--	--
Glutaric acidemia type II	--	X	--	--	--	--
Medium-chain ketoacyl-CoA thiolase deficiency	--	X	--	--	--	--
2,4 Dienoyl-CoA reductase deficiency	--	X	--	--	--	--
Carnitine palmitoyltransferase type I deficiency	--	X	--	--	--	--
Carnitine palmitoyltransferase type II deficiency	--	X	--	--	--	--
Carnitine acylcarnitine translocase deficiency	--	X	--	--	--	--
Argininemia	--	--	X	--	--	--
Citrullinemia, type II	--	--	X	--	--	--
Hypermethioninemia	--	--	X	--	--	--
Benign hyperphenylalaninemia	--	--	X	--	--	--
Biopterin defect in cofactor biosynthesis	--	--	X	--	--	--
Biopterin defect in cofactor regeneration	--	--	X	--	--	--

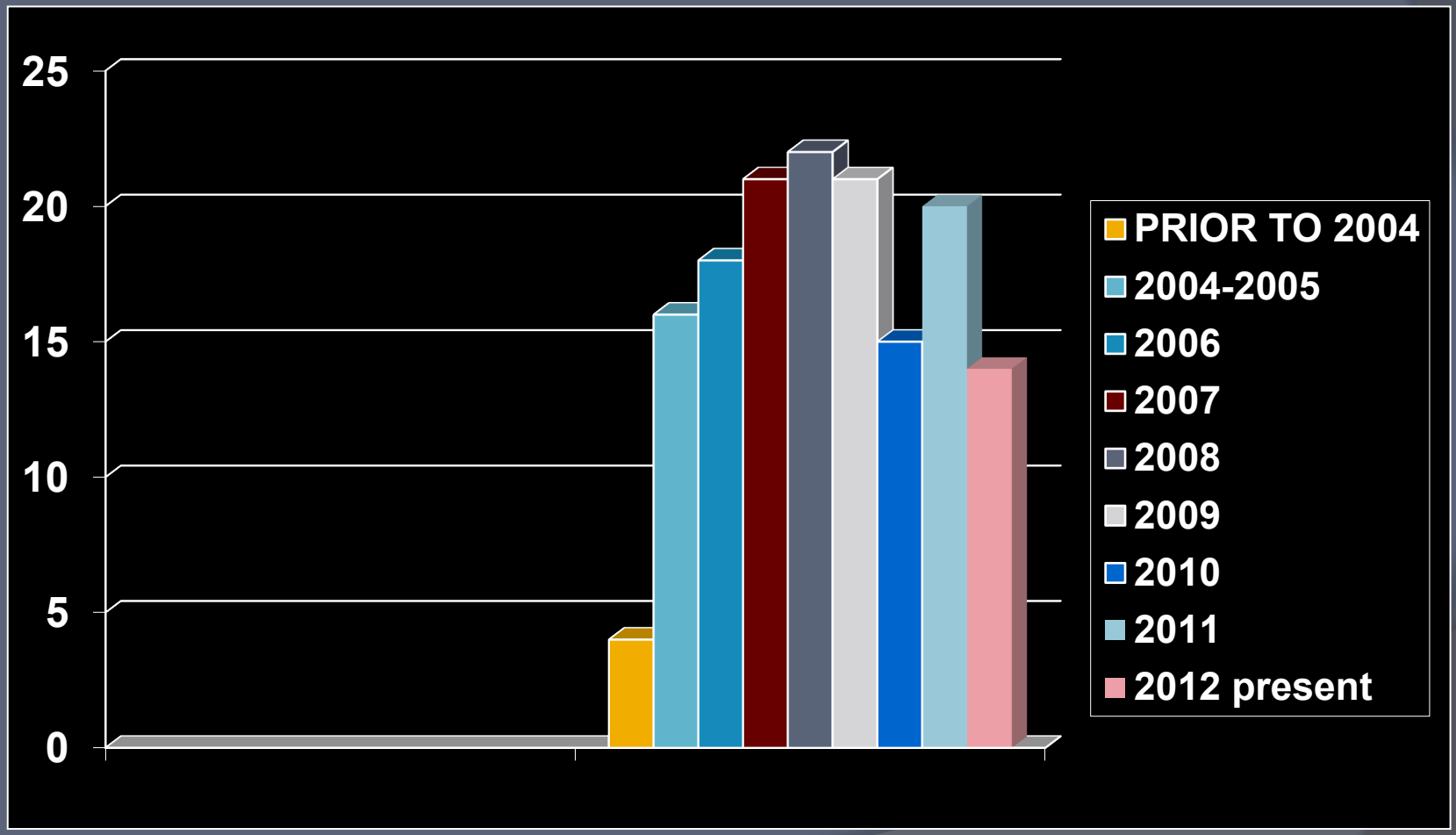
Secondary Condition – Continued	Metabolic Disorder - Organic acid condition	Metabolic Disorder - Fatty acid oxidation disorder	Metabolic Disorder - Amino acid disorder	Endocrine Disorder	Hemoglobin Disorder	Other Disorder
Tyrosinemia, type II	--	--	X	--	--	--
Tyrosinemia, type III	--	--	X	--	--	--
Various other hemoglobinopathies	--	--	--	--	X	--
Galactoepimerase deficiency	--	--	--	--	--	X
Galactokinase deficiency	--	--	--	--	--	X
T-cell related lymphocyte deficiencies	--	--	--	--	--	X

Conditions Screened* by State



*Core and secondary conditions

Alabama NBS: New Diagnoses Since Initiation of Expanded Newborn Screening



Diagnostic frequency approx. 1/3000

Lane Rutledge, MD

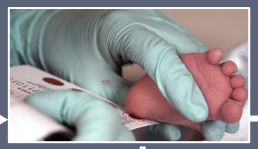
Overview of a Modern Newborn Screening Workflow

Follow-up testing

- Required to confirm or refute screening results
- Vary significantly by state
- Most infants (90%) with abnormal NBS results have normal follow-up
  - Prematurity
  - TPN or certain formulas
- If disease is confirmed then treatment is initiated immediately

Start

Sample collection by heel stick at 24 – 48 hrs



Guthrie card

Transport to NBS program

Screen positive

Screen negative

Invalid sample
- unsat
- sample <24 hrs
- delivered >14 days
- TPN or transfusion
- prematurity

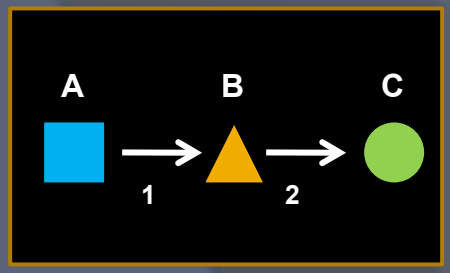
Physician immediately contacted by phone

Results sent to referring physician

Repeat sample requested

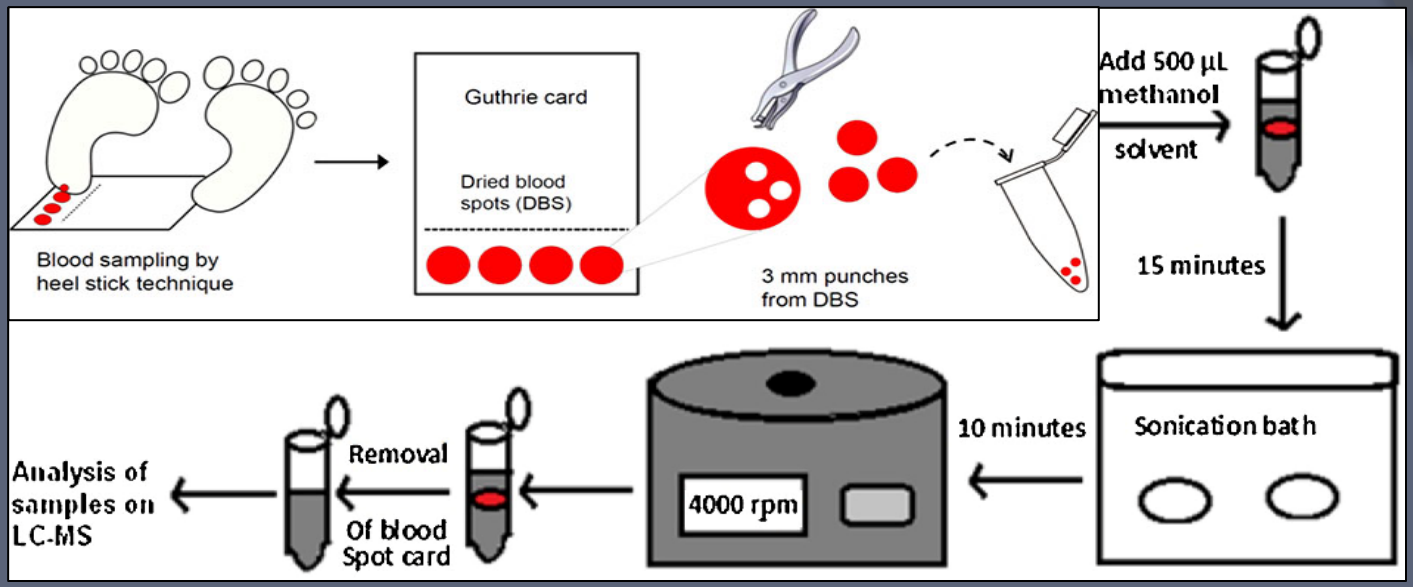
Referral for follow-up to confirm diagnosis and begin treatment

Analysis of Metabolites



- Small molecule substrates or products of enzyme-catalyzed reactions
  - Targeted metabolomics
    - Biomarkers
  - Precise instrumental analysis techniques
  - Accurate and appropriate reference ranges
    - Caution: overreliance on ref ranges
  - Quality control extremely important

Blood Spot Sample Preparation



A. Punch out one spot from Guthrie card (typically 3/16" or 3mm).

B. Add 100 µL MeOH (with internal standards) and extract for 30 minutes

C. Transfer supernatant into second plate.

D. Evaporate to dryness under nitrogen with mild (40°C) heating.

E. Add 100 µL 3 N Butanolic HCl to each sample and heat at 60°C for 15 minutes for butylation.

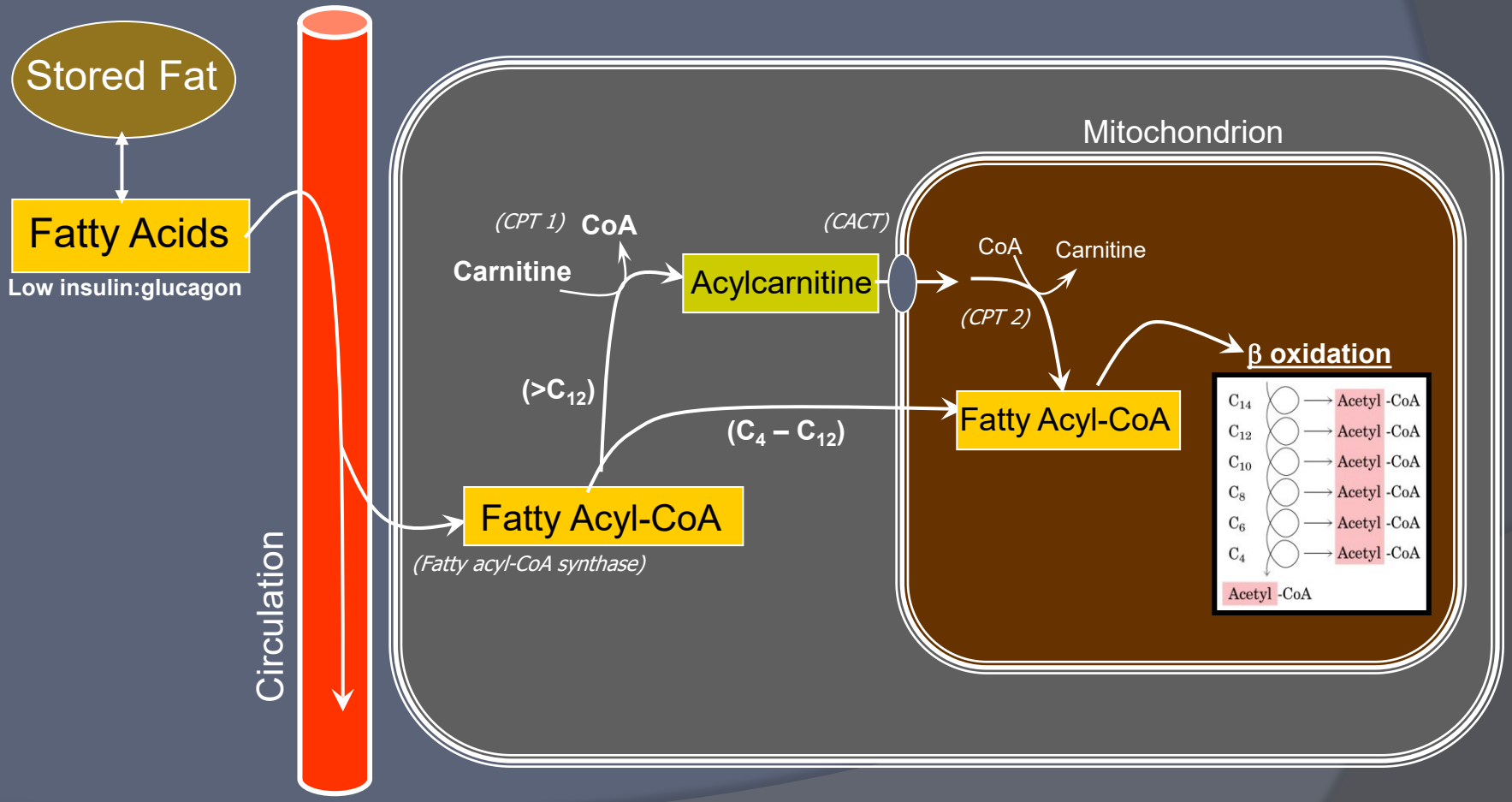
F. Evaporate to dryness under nitrogen with mild (40°C) heating.

G. Add 100 µL 80% MeCN to dissolve each sample.

H. Inject 10 µL into mobile phase

https://www.semanticscholar.org/paper/LC-MS%2FMS-determination-of-pramipexole-on-rat-dried-Rao-Sravan/774eaf32452499b53bbc29b0311c5da0dbe403ad/figure/0
https://www.piv.or.kr/ViewImage.php?Type=F&aid=596383&id=F1&afn=1153_PIV_24_3_134&fn=_1153PIV

Acylcarnitines: Intermediates of Fatty/Organic Acid Oxidation



Acylcarnitines as Biomarkers

- Deficient fatty/organic acid oxidation results in accumulation of one or more size-specific acylcarnitines in blood
  - Effectively measured via MSMS
- Initial basis for expanded newborn screening
- Disorders detected
  - Fatty acid oxidation disorders
  - Organic acid disorders
  - Other conditions identified
    - Ketosis, acidosis, catabolism, liver disease, renal disease, MCT feeding, etc
- Methodology
  - MSMS analysis of butylated acylcarnitines
  - Quantification of >30 acylcarnitines
  - Analytical time: ~2 hrs

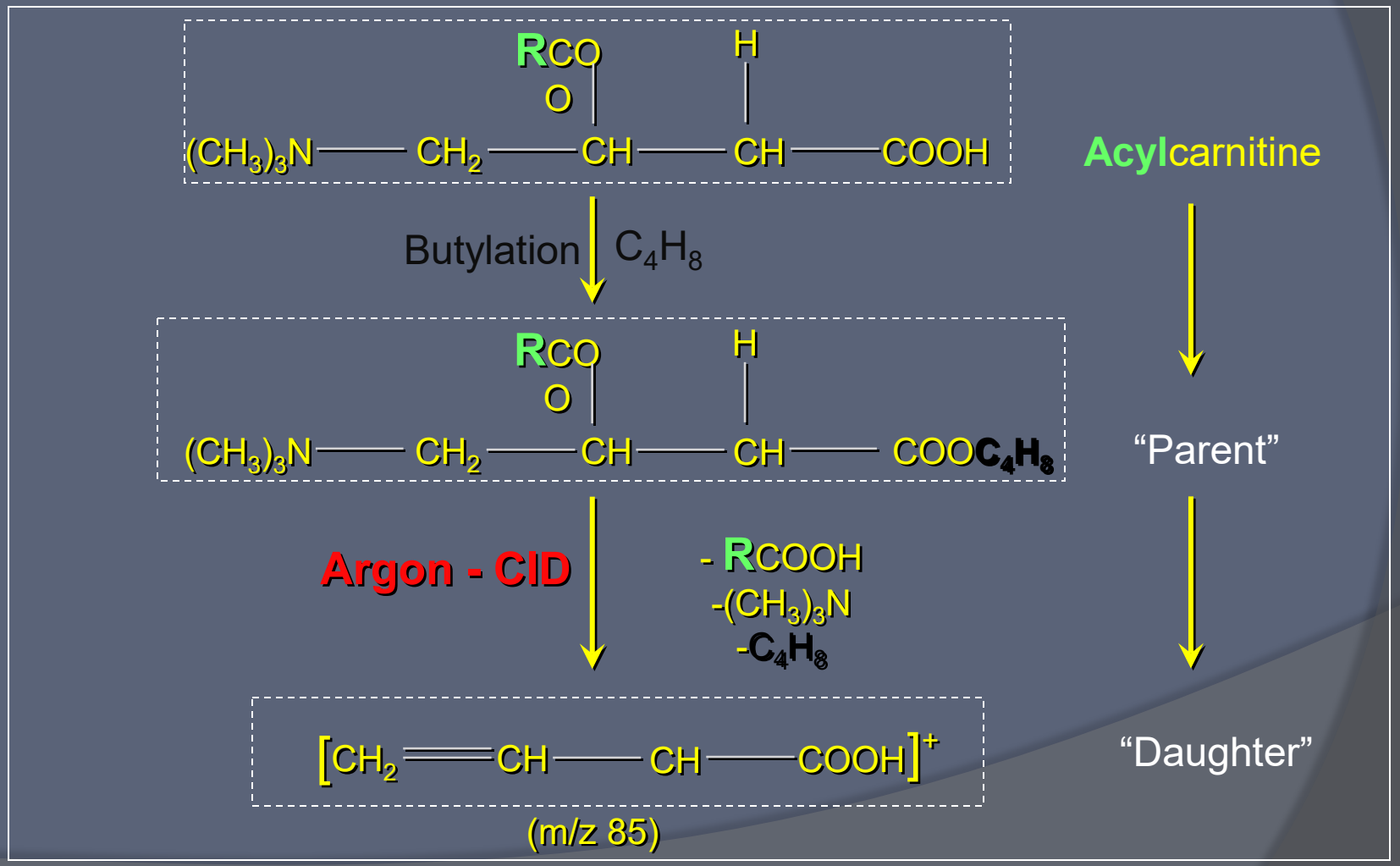
Acylcarnitine Analysis

- Sample requirements
  - Plasma (≥1 mL)
    - 20 ul used in assay
- Limitations
  - Interfering substances
  - Results generally not considered to be diagnostic (enzyme activity and/or sequence analysis)
- Confounders
  - Liver/kidney disease (AC-DCs)
  - Ketosis (C2, C4-OH, C12:1, C14:1)
  - MCT oil (C8, C10)
  - Valproate (C0, C8, C10)
  - Carnitine supplements (short chain ACs)
  - Cefotaxime (C14:1, C16:1-OH)
  - Cheese (C3)

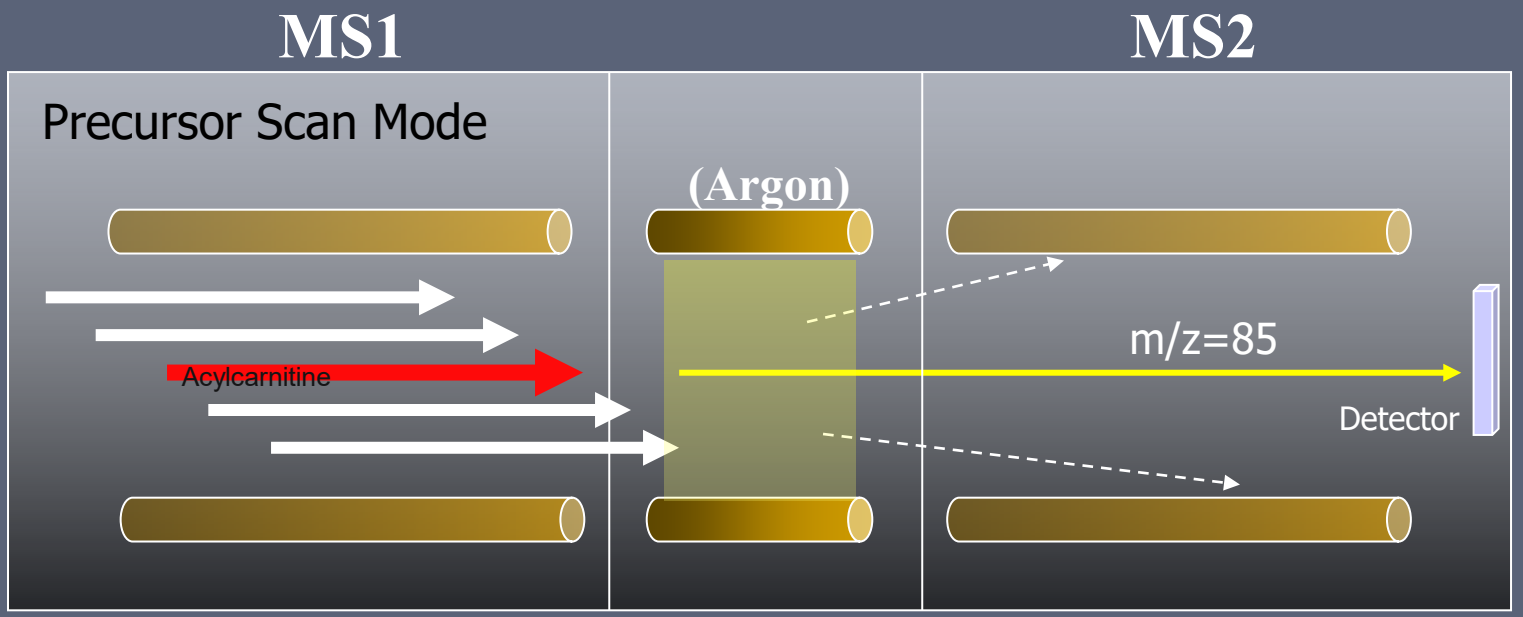
Waters Flow-Injection Triple Quadrupole Tandem Mass Spectrometer

https://www.waters.com/waters/en_US/RenataDX-Screening-System/nav.htm?cid=134986073&locale=en_US

Acylcarnitines: Derivatization and Fragmentation



Precursor Analysis of Plasma Acylcarnitines ("Parents of 85")

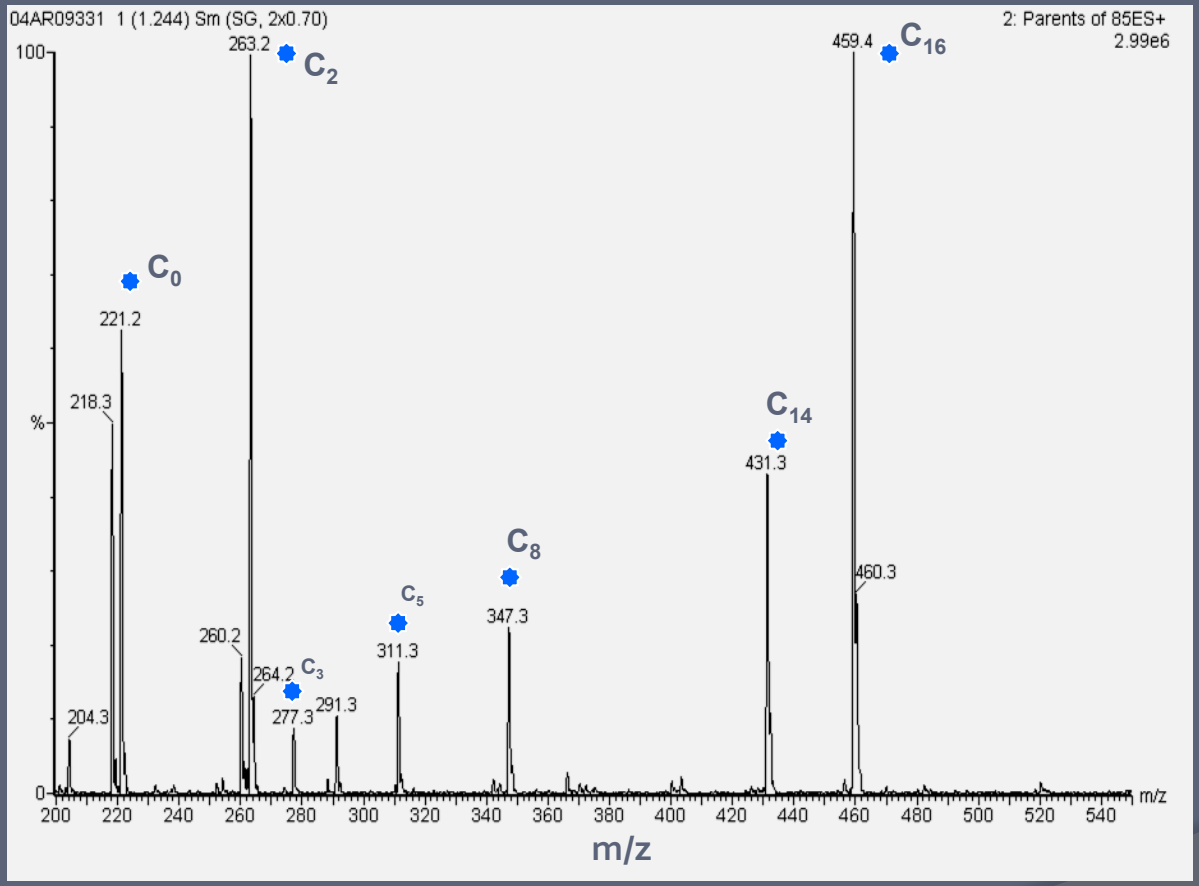


Scanning:
-sequential passage of all masses

CID

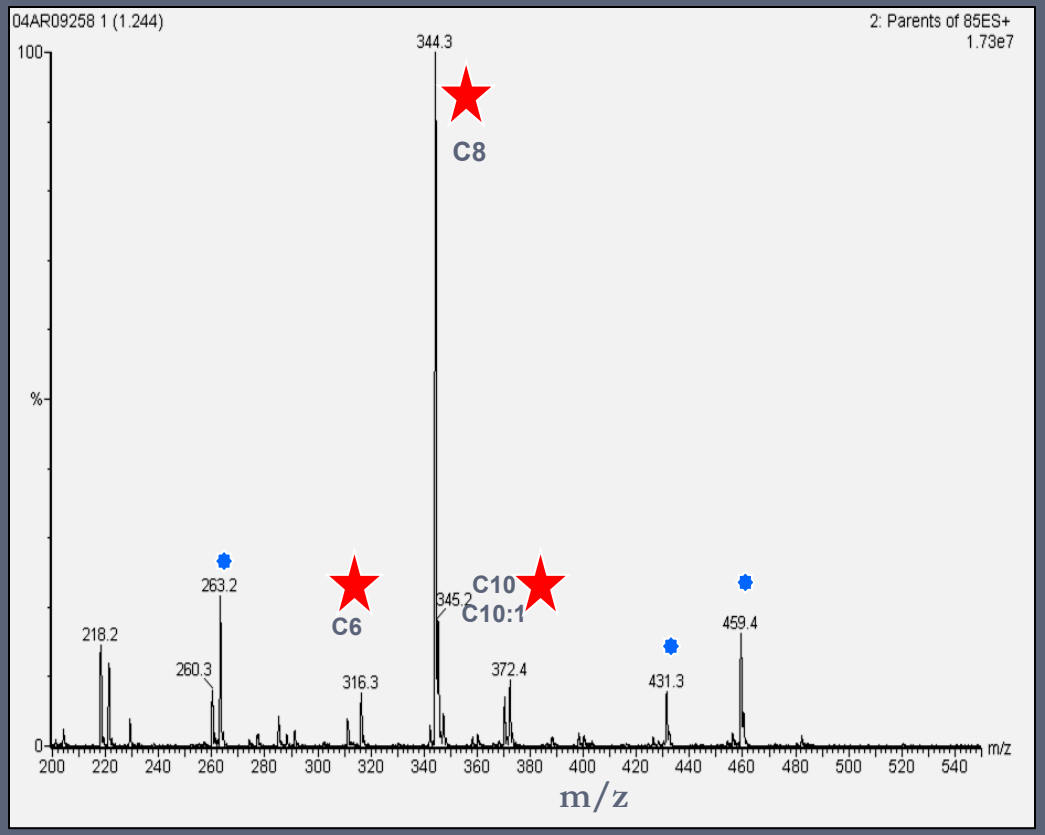
Static
-specific daughter mass only; refer back to parent precursor of m/z=85

Normal Acylcarnitine Profile Chromatogram



● = internal standard peak

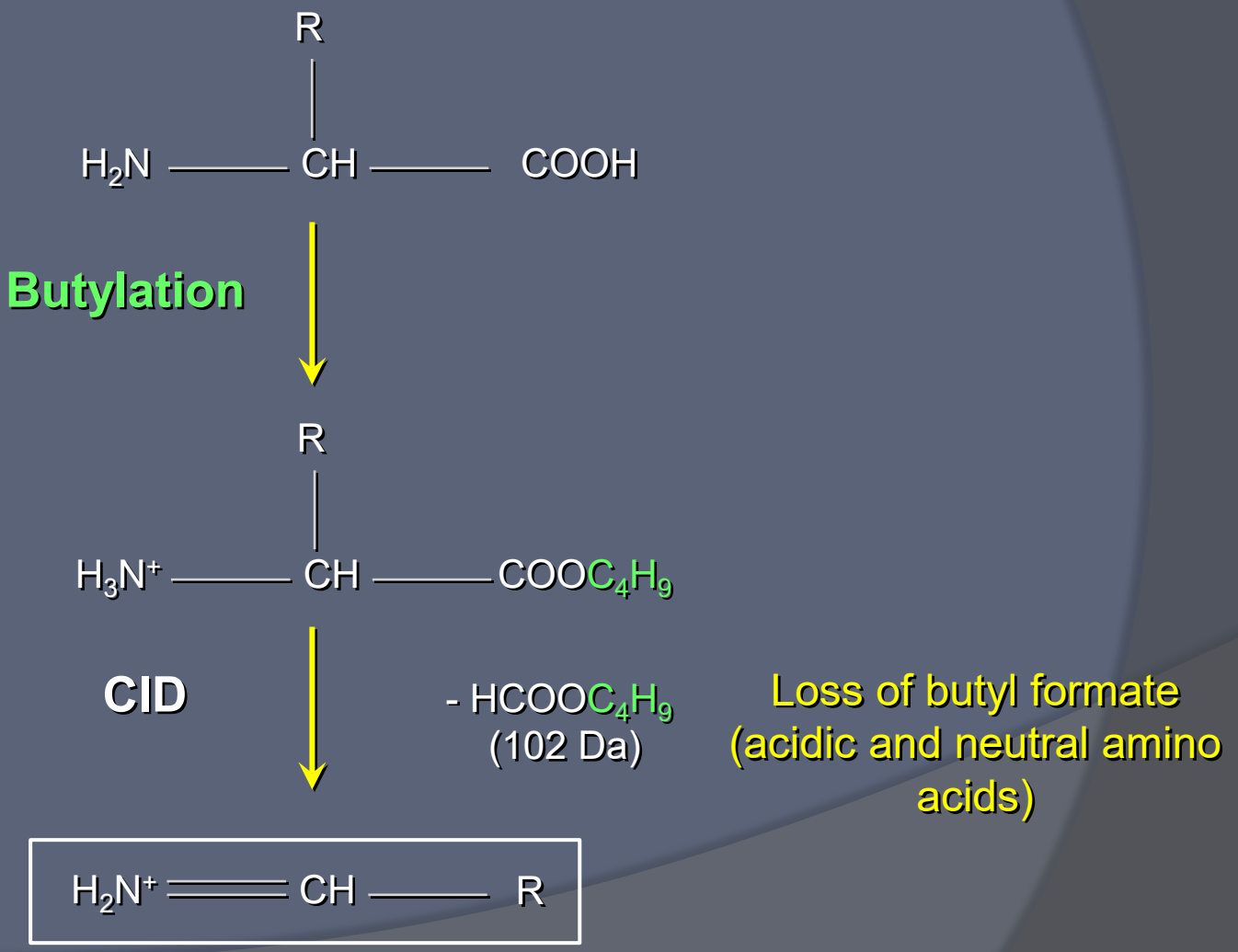
Abnormal Acylcarnitine Profile: MCAD Deficiency



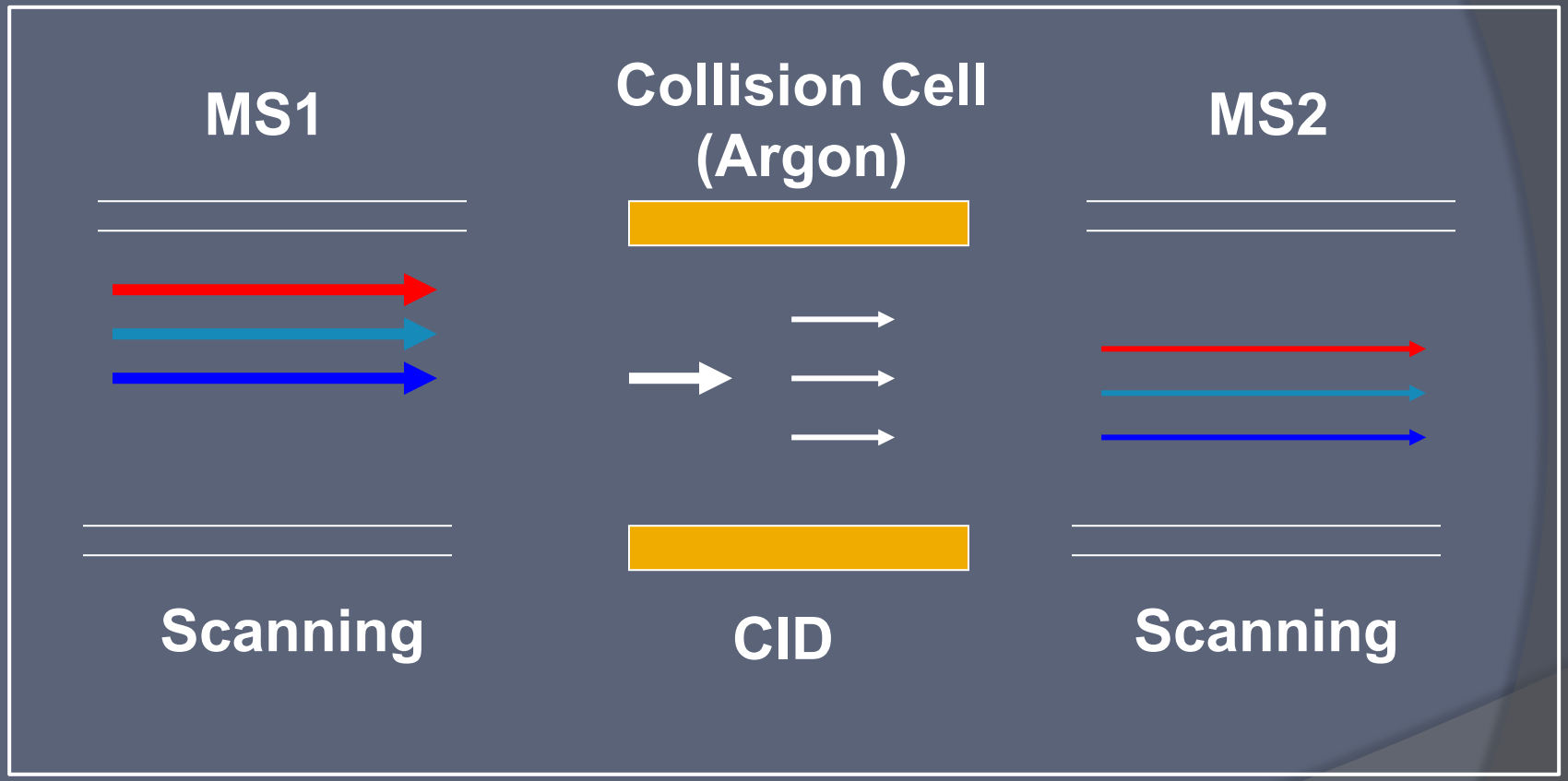
MCAD Deficiency

- Medium Chain Acyl-CoA Dehydrogenase (MCAD) deficiency
- Most common defect of mitochondrial FAO (1:12,000)
- Lethargy, seizures, hypoketotic hypoglycemia, sudden death
- Diagnosis allows for treatment (avoidance of fasting)
  - Clinical utility

Amino Acids: Derivatization and Fragmentation

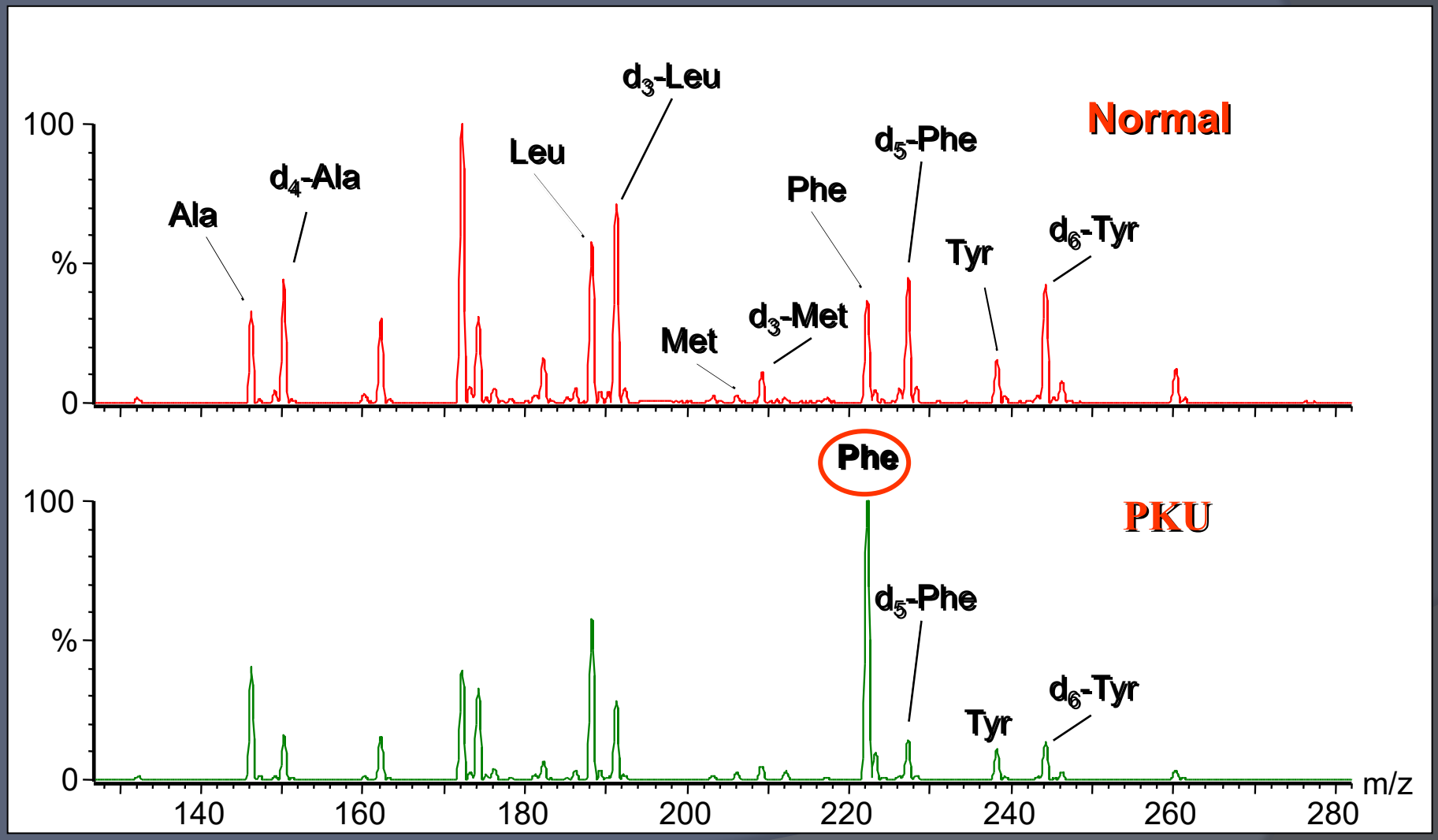


Neutral Loss Scan for Amino Acids



- Loss of 119 Da for basic amino acids
- Loss of 102 Da for acidic and neutral amino acids

Phenylketonuria (PKU)



Benefits of Newborn Screening

- Improved health outcomes:
  - 4000 – 5000 newborns/yr experience significantly improved health outcomes[1]
  - prevents diagnostic odysseys
- Cost-effectiveness (congenital hypothyroidism):
  - Annual economic cost of screening and early treatment for CH is 20-fold less than treating severely affected patients who were not screened
    - ($20 million vs $400 million)[2]

1. http://www.councilforresponsiblegenetics.org/genewatch/GeneWatchPage.aspx?pageId=450#endnotes

2.CDC. MMWR 2004; 53(3):57–59
Grosse SD. AERE Newsletter. 2007; 27(2):17-21Grosse, SD et al. Med Care. 2009; 47(7 Suppl1):S94–S103

Limitations of NBS

- False positives
- False negatives
- Many types of metabolic disorders are not screened
- Questionable clinical utility for some screened disorders
- Lack of clinical and laboratory expertise
- Significant financial constraints

False Positives and False Negatives

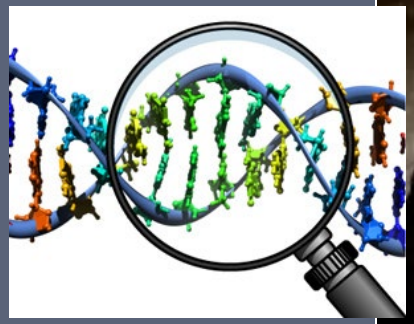
- False positives (positive result/disease absent)
  - Create significant stress for families
  - Causes:
    - Lab error, prematurity, diet (MCT oil/MCAD), sample handling (frozen blood), sample handling (heat inactivation of GALT), sample contamination (bacteria)
  - Rates:
    - General FP range: 0.01 – 1.5% (variable; not widely reported)
      - 10 – 1500 false positives/100,000 births
      - >90% of all abnormal NBS results ultimately unaffected
  - Second tier testing:
    - Reflex follow-up testing done in-house for some conditions in some states, w/o need for additional clinical visit
- False negatives (negative result/disease present)
  - Causes:
    - Lab error, blood transfusion (Galactosemia), mild variants, test done too soon (maternal effects), sample storage
  - Rates:
    - Usually very low (not widely reported)
    - Pilot study: up to 1% of patients with moderate congenital adrenal hyperplasia (steroid hormone dysfunction) would have been missed using an older method*

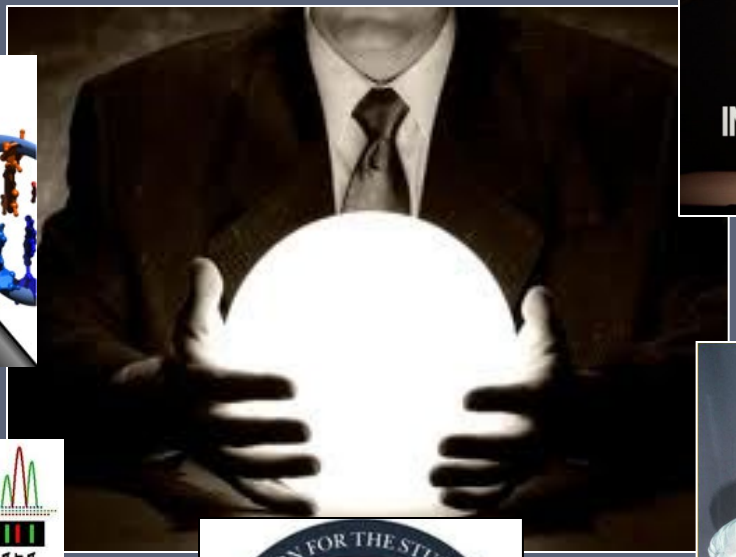
*Eur J Endocrinol. 2005 Jun;152(6):869-74.

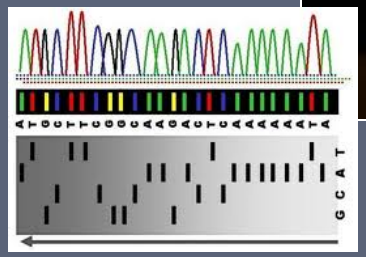
Newborn Screening: Ethical Issues

- Privacy
  - Sample retention and security of stored data
- Clinical utility is questionable for some screened disorders
  - Severe forms of certain disorders that may present before NBS results are available
  - Very rare disorders with small numbers of affected patients, making outcomes uncertain
  - Very mild, ill-defined phenotypes
  - Lack of treatment options

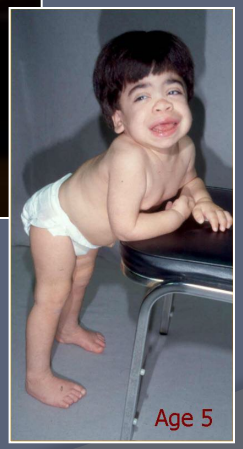
The Future of Newborn Screening







Variants of unknown significance

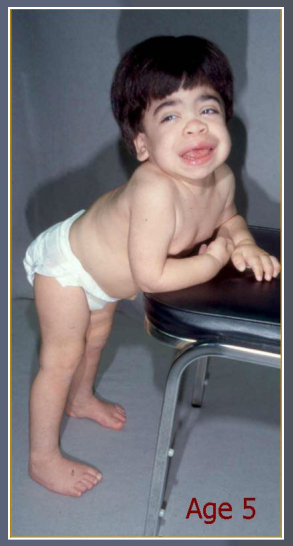


Genzyme
Google images

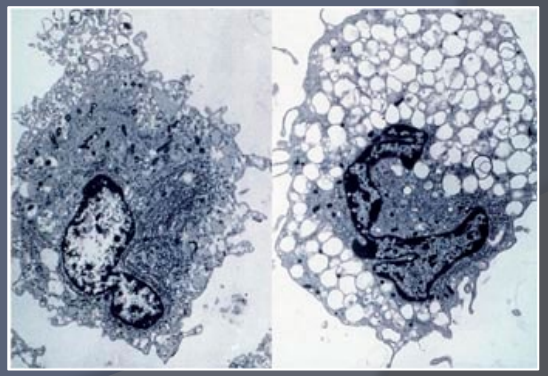
Where Does NBS Go From Here?

- The existing NBS model continues to evolve
  - More conditions being added or considered for screening (eg, LSDs)
  - Changes to current screening criteria proposed
- Next generation DNA sequencing: the new screening paradigm?
  - Potential for massive expansion of genetic screening

Newborn Screening for Lysosomal Storage Disorders (LSDs)

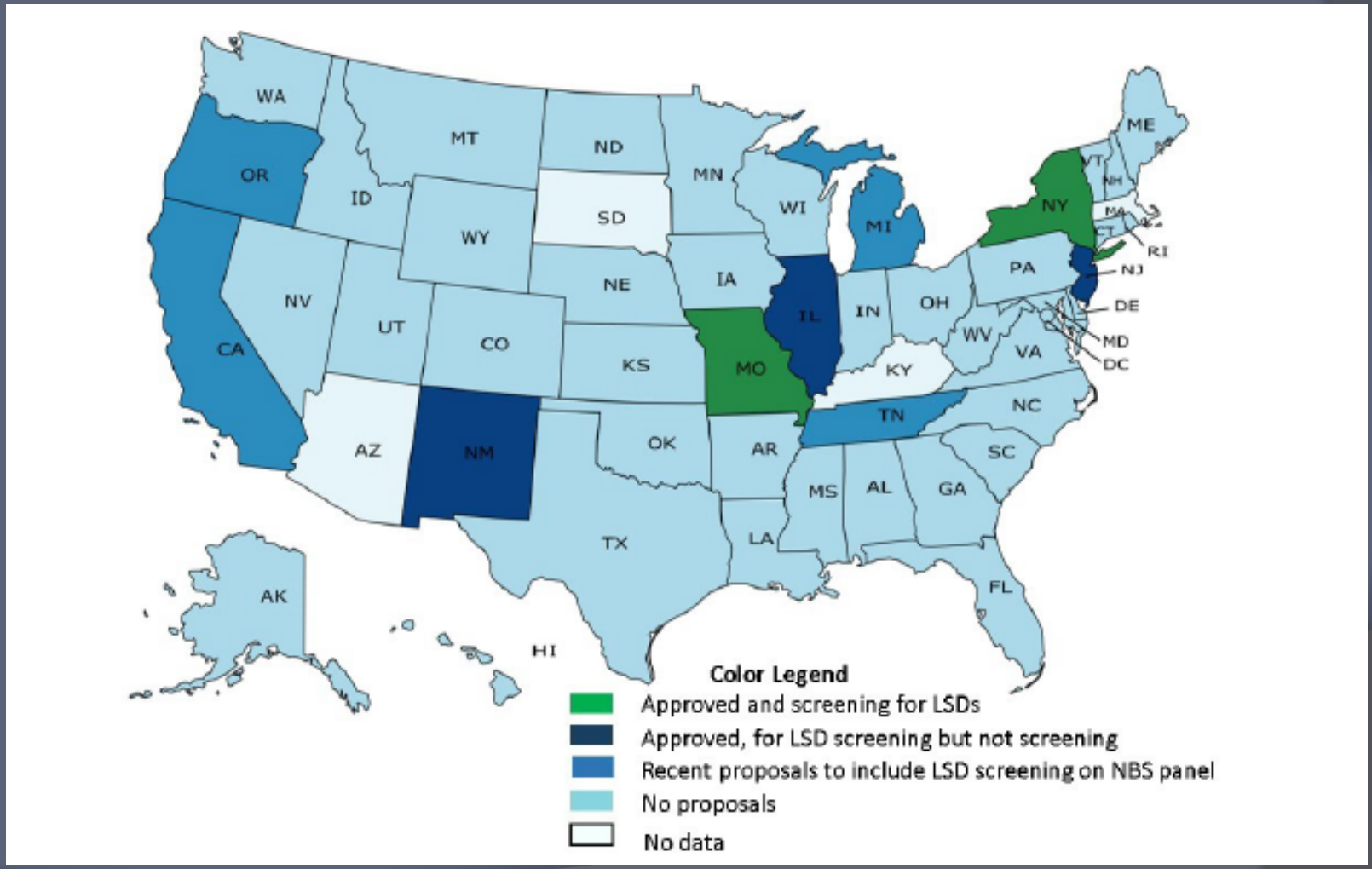


- LSDs: disorders of lysosomal enzymes that degrade/recycle cellular waste products.
- Accumulating materials cause progressive damage to multiple organs, incl CNS
  - Often early mortality w/o treatment
- Estimated incidence: 1:5000 – 10,000
- LSDs as candidates for NBS:
  - Usually not apparent at birth
  - Diagnosis is often delayed
  - Growing number of therapeutic options and demonstrated benefits of early treatment
  - Multiplex screening methods now available
- Several programs now offering or piloting limited LSD screening (Alabama 2023: MPS 1, Pompe)



Genzyme

Screening for Lysosomal Storage Disorders (2017)



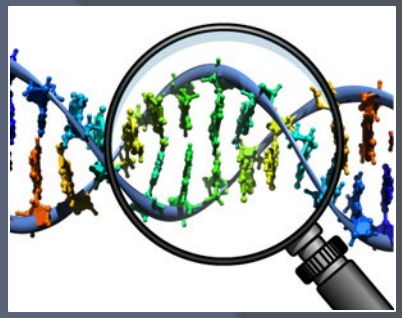
Lockande et al (2017) J Rare Disord 5, 21-30

Should We Screen for Diseases Without an Effective Therapy?

- Cornerstone of traditional screening: must be an effective treatment available
- However, it has been suggested that future screening should consider other benefits:
  - avoiding diagnostic odysseys
  - making preparations for disease
  - reproductive decisions
  - early access to promising new therapies

Alexander and van Dyck, 2006
Tarini 2008

The Next Big Thing: Next Generation Sequencing (NGS)?



- DNA sequencing-based methods may represent the future of genetic screening
- Will initially take the form of small scale, targeted panels
  - The National Institute of Child Health and Human Development (NICHD) is currently funding efforts to develop DNA-based screening.
- Ultimately, the entire genome of all newborns may be routinely sequenced at birth
- Paradigm shift? Functional (biochemical) testing to confirm molecular screening (see below)

Obstacles to NGS Screening

- Cost
  - Must be cost effective: current NBS testing costs ~$2.00/disorder. Current genome sequencing costs about $1000
    - Costs are falling rapidly; may become cost-effective in the next 5 – 10 years
- Infrastructure
  - Bioinformatics: data storage and analysis
  - Expansion of follow-up programs?
  - Genetic counseling
- Ethical considerations
  - Security/privacy
  - Variants of unknown significance
  - Incidental findings

nature medicine

LETTERS

https://doi.org/10.1038/s41591-020-0966-5

Check for updates

The role of exome sequencing in newborn screening for inborn errors of metabolism

Aashish N. Adhikari[1,2] ✉, Renata C. Gallagher[2,3], Yaqiong Wang[1], Robert J. Currier[3], George Amatuni[3], Laia Bassaganyas[2], Flavia Chen[2,4], Kunal Kundu[1,5], Mark Kvale[2], Sean D. Mooney[6], Robert L. Nussbaum[2,7], Savanna S. Randi[8], Jeremy Sanford[8], Joseph T. Shieh[2,3], Rajgopal Srinivasan[5], Uma Sunderam[5], Hao Tang[9], Dedeepya Vaka[2], Yangyun Zou[1], Barbara A. Koenig[2,4], Pui-Yan Kwok[2,10,11], Neil Risch[2,12], Jennifer M. Puck[2,3,10,13,16] ✉ and Steven E. Brenner[1,2,14,15,16] ✉

- WES vs MSMS
  - Sensitivity
    - WES: 93.7%
    - MSMS: 99%
  - Specificity
    - WES: 98.4% (8000 false pos/yr/CA)
    - MSMS: >99.8% (1362 false pos/2015/CA)
- WES would be insufficient for NBS, but represents a potentially effective option for reflex follow-up testing
- Also may be useful for situations where biochemical testing isn't available (eg, lack of biomarker)

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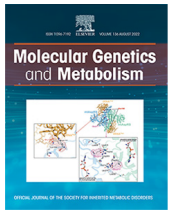

Metabolic diversity in human populations and correlation with genetic and ancestral geographic distances

Gang Peng [a,b], Andrew J. Pakstis [a], Neeru Gandotra [a], Tina M. Cowan [c], Hongyu Zhao [a,b], Kenneth K. Kidd [a], Curt Scharfe [a,*]

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[c] Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

- Demonstrates variation inherent to metabolomes across different populations
- This information may be applied to newborn screening paradigms to improve accuracy

Thank You!

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