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

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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.

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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

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What is Newborn Screening (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

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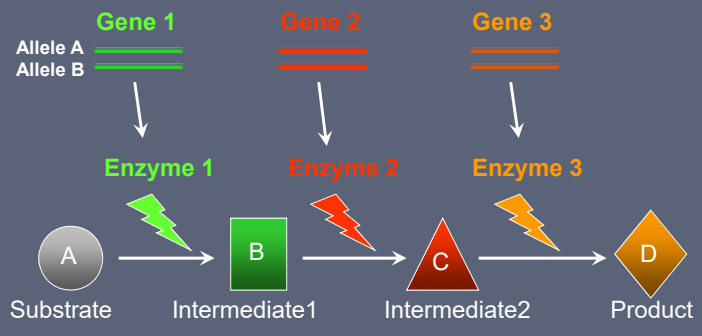
Metabolic Pathways: Sequential Enzyme-catalyzed Reactions



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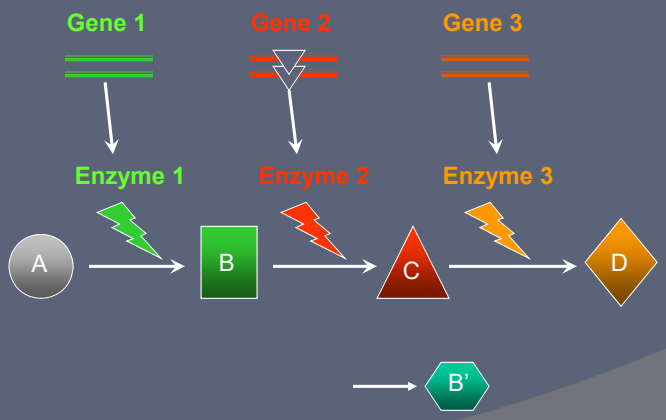
Inherited Metabolic Disorders: Recessive Metabolic Enzyme Dysfunction



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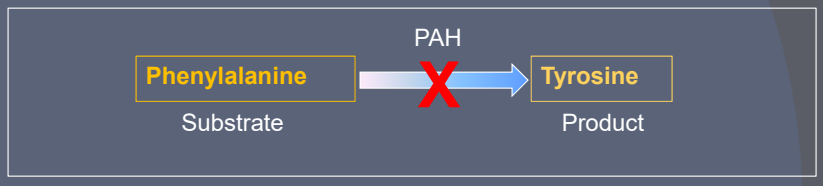
The Origins of NBS: Phenylketonuria (PKU)



- 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

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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

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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

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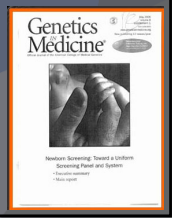
A Brief History of Newborn Screening: the Era of Mass Spectrometry

- 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



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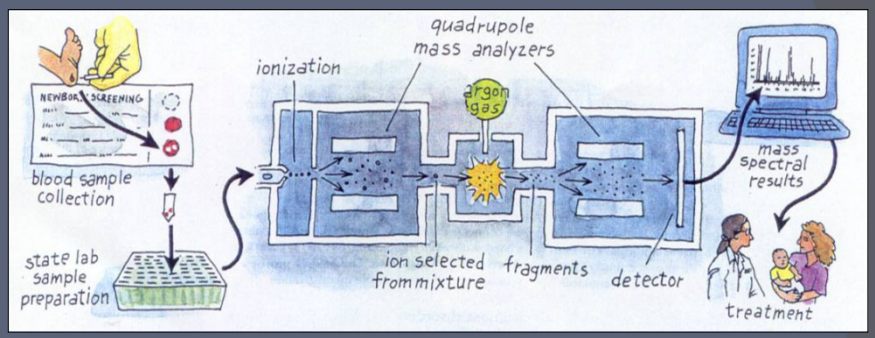
Modern Newborn Screening via Tandem Mass Spectrometry



Blood sample collected 24 – 48 hrs after birth (may be follow-up screen at 2 – 4 weeks)

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

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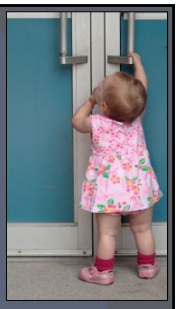
Criteria for Inclusion in the ACMG ENS Core Screening Panel (2006)



- 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

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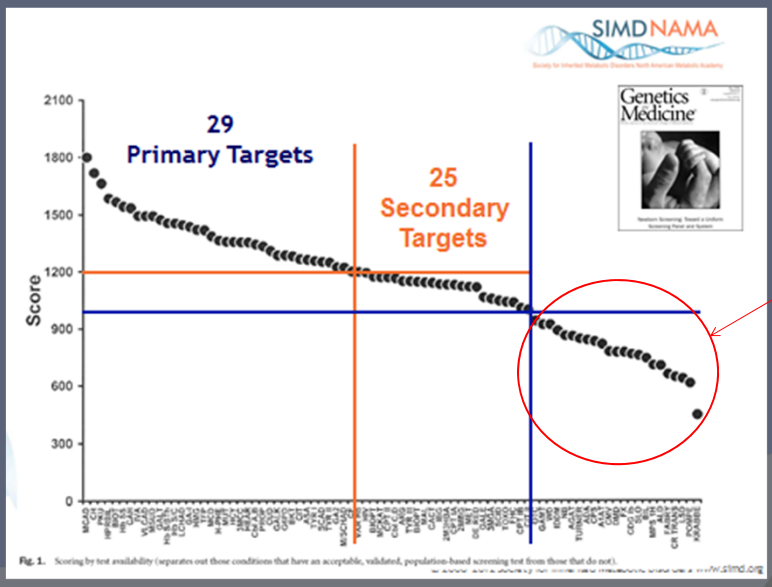
2005 ACMG Panel Scores



LSDs now being added to many NBS panels

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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)

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HRSA Recommended Uniform Screening Panel (RUSP) 2018

Recommended Uniform Screening Panel
Core Conditions
(As of July 2018)

Core Condition	Metabolic Disorder			Endocrine Disorder	Hemoglobin Disorder	Other Disorder
	Organic acid condition	Fatty acid oxidation disorder	Amino acid 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, ßeta-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
Severe Combined Immunodeficiencies						X
Mucopolysaccharidosis Type 1						X
X-linked Adrenoleukodystrophy						X
Spinal Muscular Atrophy due to homozygous deletion of exon 7 in SMN1						X

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2018 RUSP Secondary Conditions

Recommended Uniform Screening Panel[1]
SECONDARY[2] CONDITIONS [3]
(As of July 2018)

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

1. Selection of conditions based upon "Newborn Screening: Towards a Uniform Screening Panel and System." *Genetic Med.* 2006; 8(5) Suppl: S12-S252" as authored by the American College of Medical Genetics (ACMG) and commissioned by the Health Resources and Services Administration (HRSA).
2. Disorders that can be detected in the differential diagnosis of a core disorder.
Nomenclature for Conditions based upon "Naming and Counting Disorders (Conditions) Included in Newborn Screening Panels." *Pediatrics.* 2006; 117 (5) Suppl: S308-S314.

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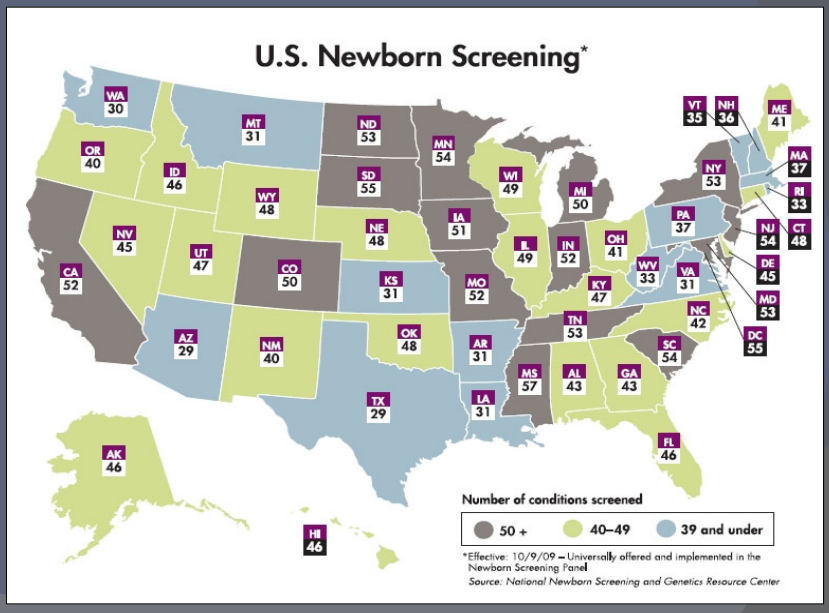
Conditions Screened* by State



*Core and secondary conditions

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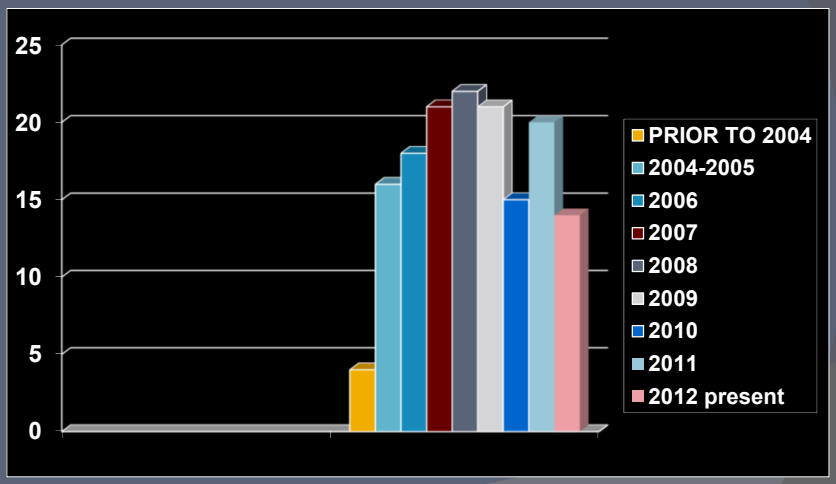
Alabama NBS: New Diagnoses Since Initiation of Expanded Newborn Screening



Diagnostic frequency approx. 1/3000

Lane Rutledge, MD

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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

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

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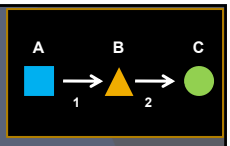
Analysis of Metabolites



- 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

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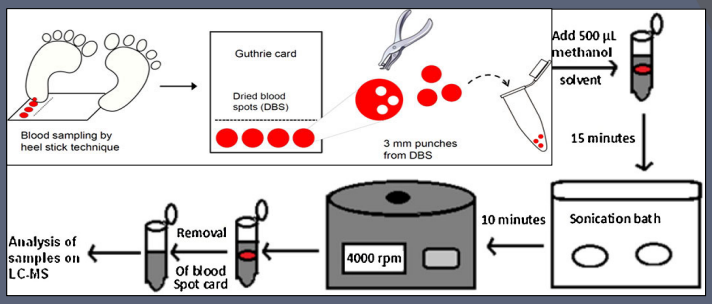
Blood Spot Sample Preparation



A. Punch out one spot from Guthrie card (typically 3/16" or 3mm).
B. Add 100 µL MeOH (with internal standards) and extact 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.pv.or.kr/viewImage.php?Type=F&aid=590383&id=F1&afn=1153_PIV_24_3_134&fn_1153PIV

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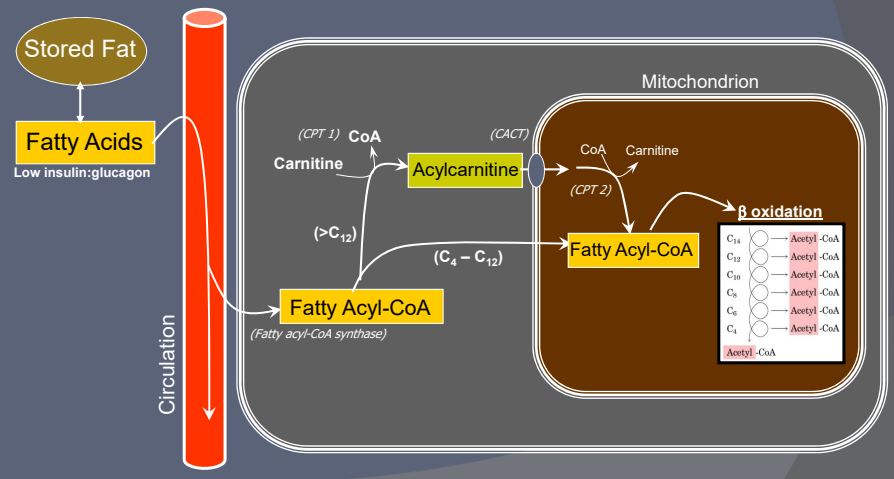
Acylcarnitines: Intermediates of Fatty/Organic Acid Oxidation



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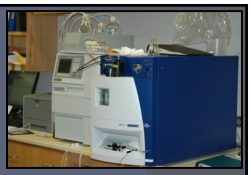
Acylcarnitines as Biomarkers



- Deficient fatty/organic acid oxidation results in accumulation of one or more size-specific acylcarnitines in blood
  - Effectively measured via MSMS
- Basis for expanded newborn screening (fatty/organic acid oxidation defects)
- 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

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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)

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Waters Quattro Micro LC-MSMS

- Triple quadrupole mass spectrometer with electrospray ionization

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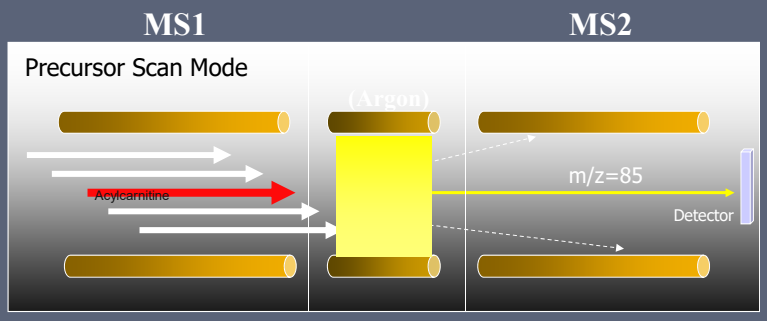
Precursor Analysis of Plasma Acylcarnitines ("Parents of 85")



Scanning:
-sequential passage of all masses

CID

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

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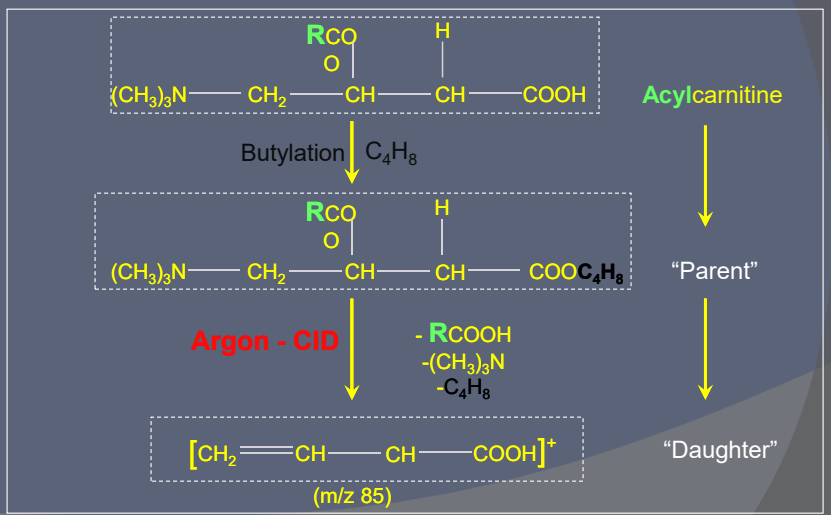
Acylcarnitines: Derivatization and Fragmentation



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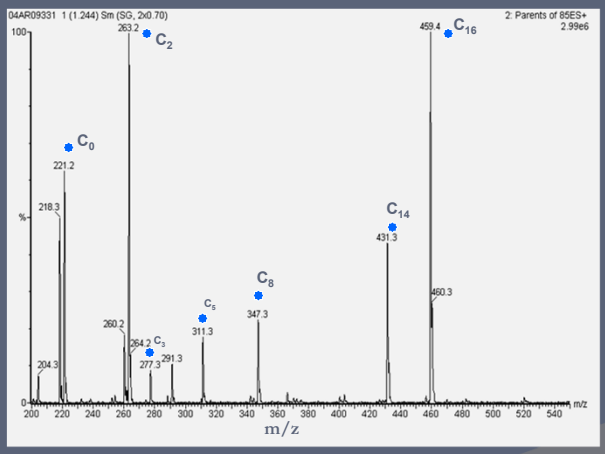
Normal Acylcarnitine Profile Chromatogram



= internal standard peak

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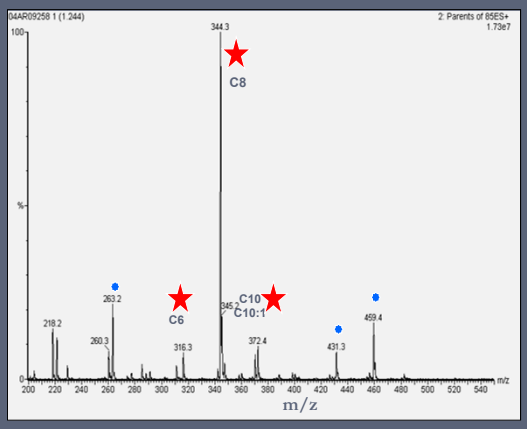
Abnormal Acylcarnitine Profile: MCAD Deficiency



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

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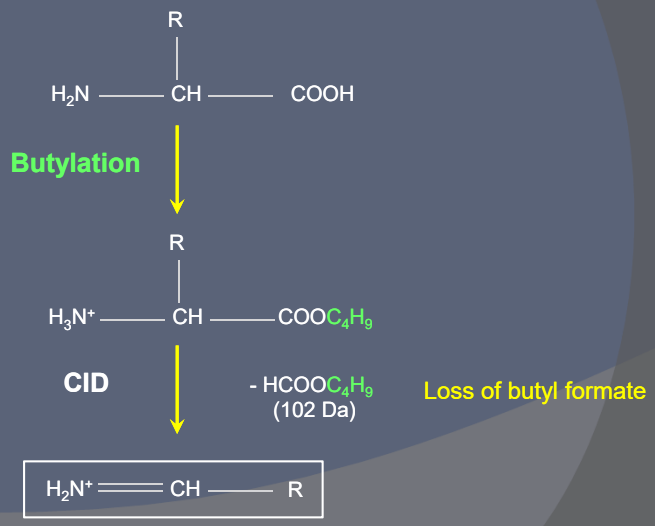
Neutral and Acidic Amino Acids: Derivatization and Fragmentation



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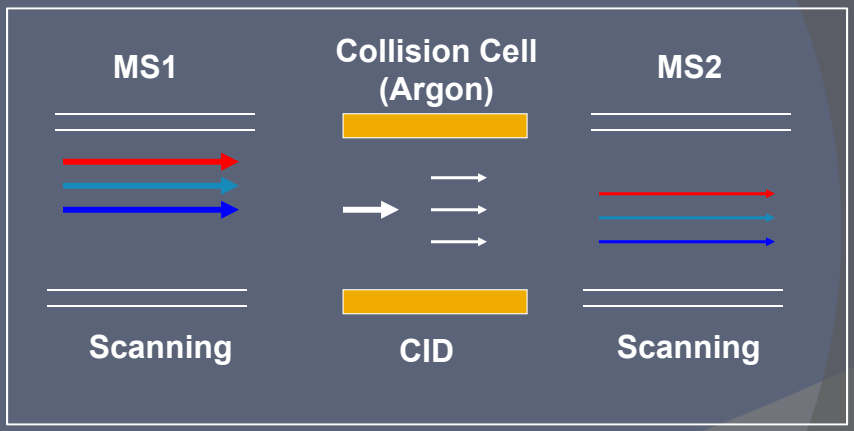
Neutral Loss Scan for Amino Acids



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

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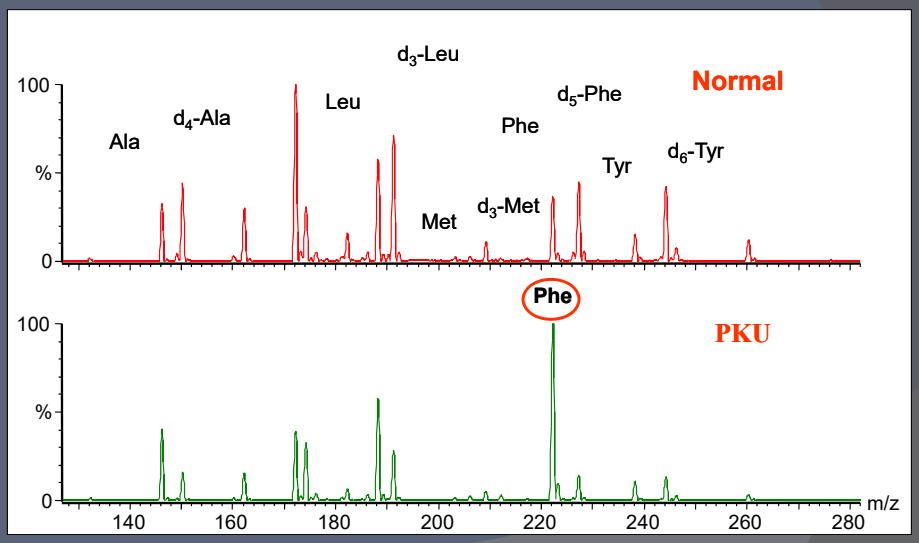
Phenylketonuria (PKU)



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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 benefit (avoiding cost of treating an affected individual) is 20fold greater than the cost of screening ($400 M vs. $20 M)[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

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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

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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.

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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

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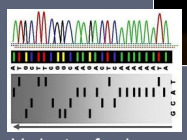
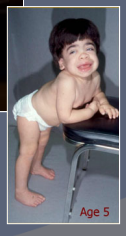
The Future of Newborn Screening



Variants of unknown significance



Genzyme
Google images

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Where Does NBS Go From Here?

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

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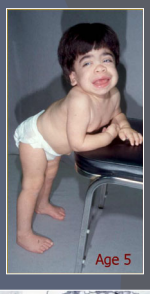
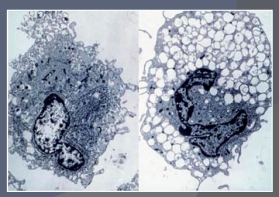
Newborn Screening for Lysosomal Storage Disorders (LSDs)



- 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: Pompe)



Genzyme

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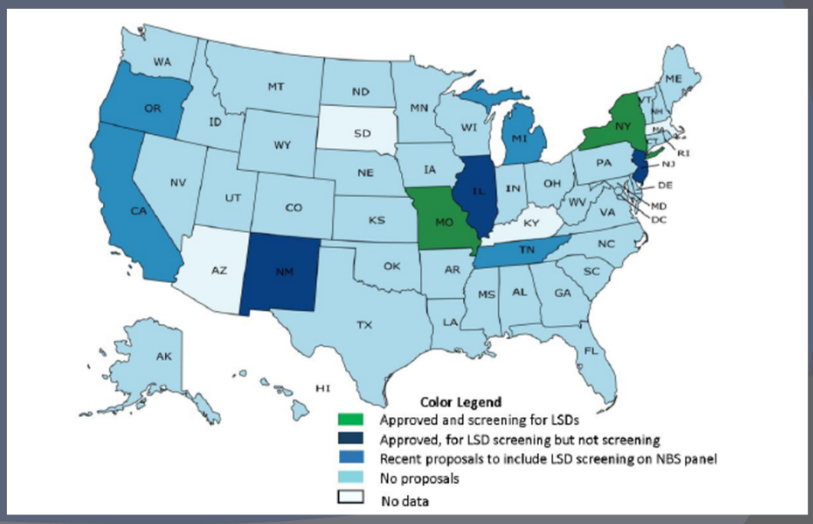
Screening for Lysosomal Storage Disorders (2017)



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

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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

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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)

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Obstacles to NGS Screening

- Cost
  - Must be cost effective: current NBS testing costs ~$2.00/disorder. Current genome sequencing costs about $1000 (w/o interpretation)
    - 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

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Thank You!

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