GBSC 724 Advanced Special Topics in Metabolomics

POPULATION-SCALE CLINICAL METABOLOMICS: EXPANDED NEWBORN SCREENING

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Lecture overview

- Introduction and relevance
- Historical perspective
- Methodology
- Future prospects

Prologue: the impact of newborn screening

- JS was born in1952 with phenylketonuria (PKU). Undiagnosed, he developed severe intellectual disability and was institutionalized at the age of 20.
- JD was born in1962 with PKU. Newborn screening was now available and led to a diagnosis at 2 weeks of age. She was placed on a special diet, and grew to be an adult with normal intelligence.

- 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 15 miles away, just across the border in a state where MCAD screening was offered. He 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.*"

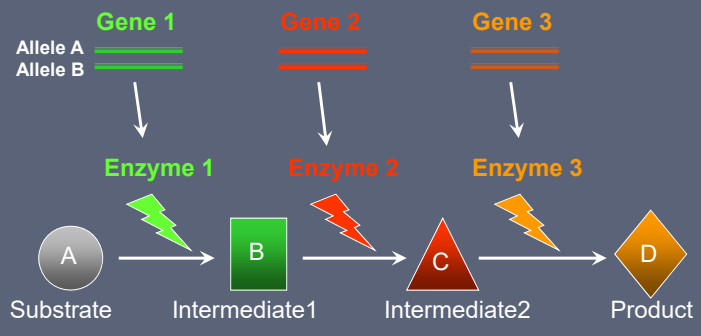
Morbidity & Mortality Weekly Report. 2011; 60(24):814-818

What is newborn screening (NBS)?

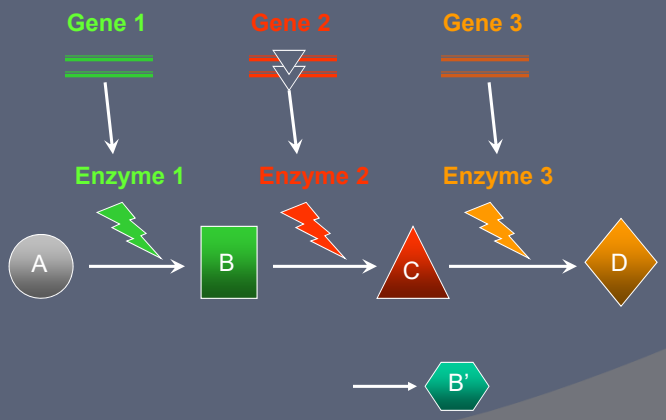
- Population scale screening of all newborns* for the presence of *treatable* conditions that are not otherwise evident at birth
  - Screening vs. diagnostic testing
- State – specific programs (no federal mandate) with significant variability
  - disorders detected
  - follow-up procedures

*USA: 4 million births/year

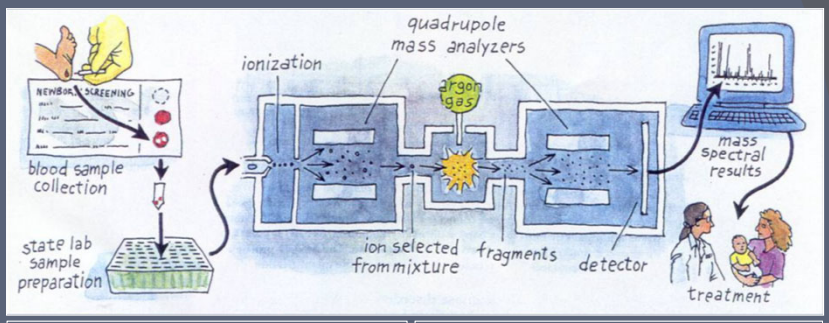
Metabolic pathways: sequential enzyme-catalyzed reactions



Inherited metabolic disorders: recessive metabolic enzyme dysfunction



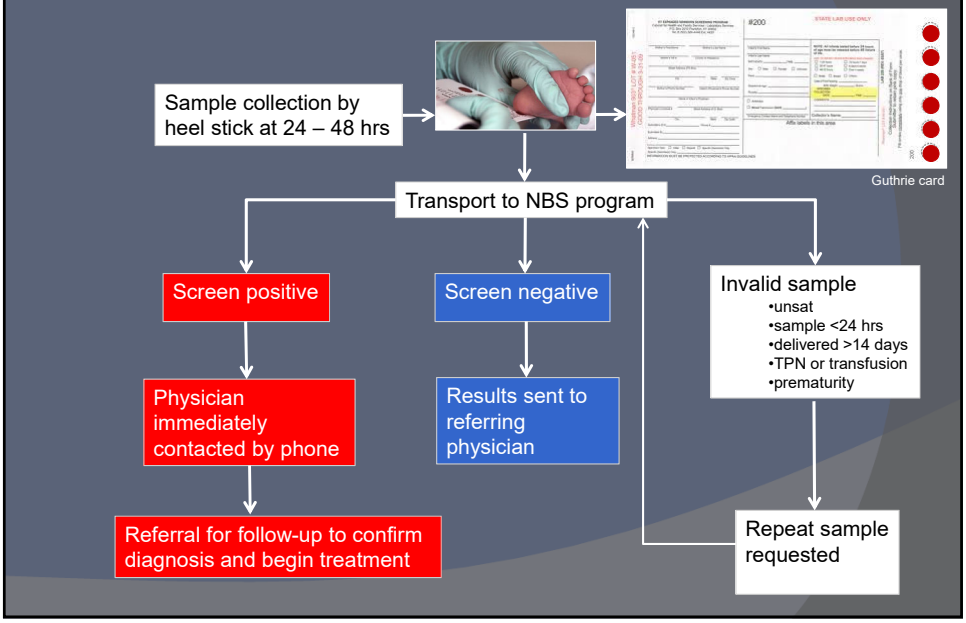
Modern newborn screening program



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

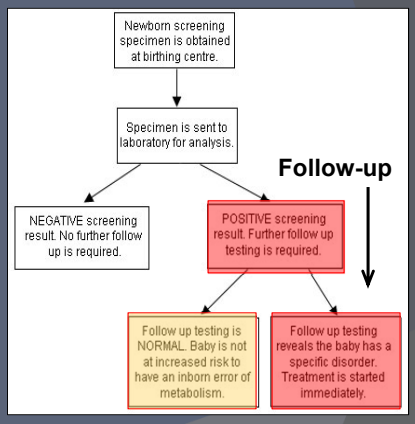
- Analytical time: ~ 5 minutes
- Metabolites detected: >20
- Conditions detected: >50

NBS: logistics and outcomes



Newborn screening follow-up programs: screening is only the beginning

- Required to confirm or refute screening results
- Follow-up programs vary significantly by state
  - Biochemical/molecular genetic laboratories
- Most infants with abnormal NBS results have normal follow-up (>90%)
  - Prematurity
  - TPN or certain formulas
- If disease is confirmed then treatment is initiated immediately



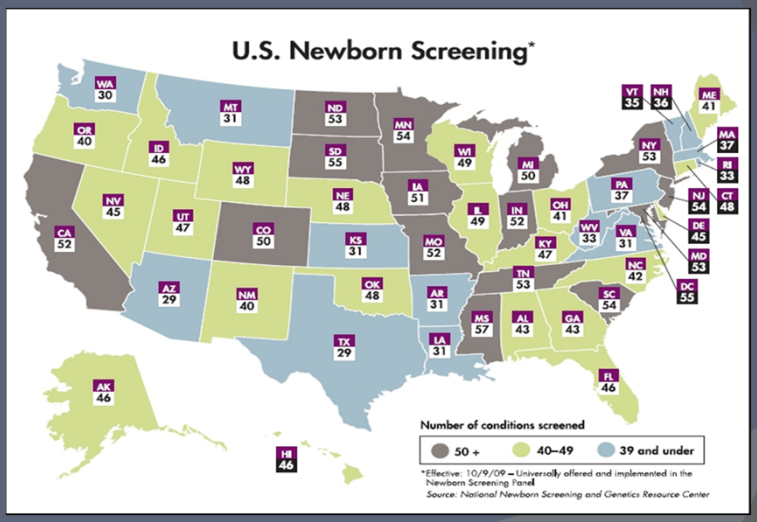
Western Australia Newborn Screening Program

Screened disorders in the United States

- Currently, 35 *core* conditions are recommended for newborn screening (2018)
  - 20 metabolic disorders (eg, PKU)
  - 2 endocrine disorders (eg, CAH)
  - 3 hemoglobin disorders (eg, sickle cell anemia)
  - 9 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
  - 24 metabolic
  - 1 hemoglobinopathy
  - 3 other

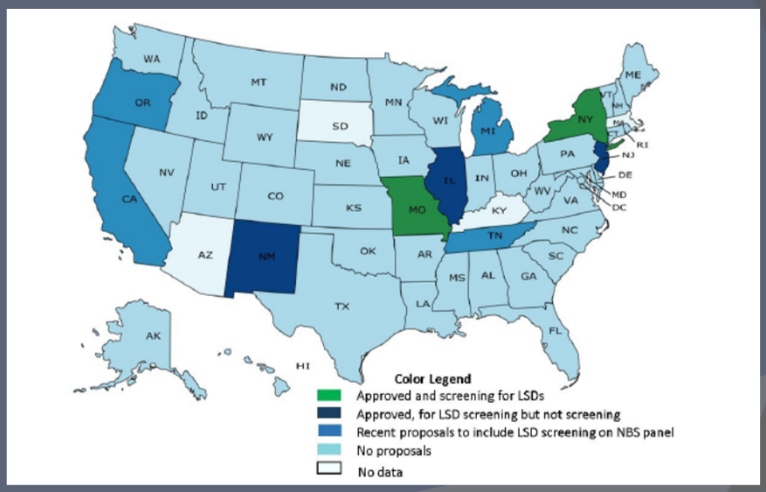
National Newborn Screening & Global Resource Center (NNSGRC)

Conditions screened* by state



*Core + secondary conditions

Screening for lysosomal storage disorders (2017)



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

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					
B-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, β-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

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.

Tangible benefits of newborn screening

- Improved health outcomes:
  - estimated that 4000 – 5000 newborns/yr experience significantly improved health outcomes as a result of early detection and initiation of treatment[1]
  - prevents diagnostic odysseys
- Cost-effective:
  - For one condition (congenital hypothyroidism) estimated annual economic benefit (eg, avoiding cost of treating an affected individual) is nearly 20 fold 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

Limitations of NBS

- False negatives
- False positives
  - Inherently low PPV when screening for multiple rare disorders
  - May create significant stress for families
- 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

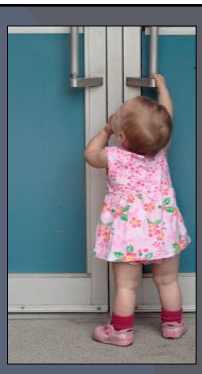
- False positives
  - Causes:
    - Lab error, diet (MCT oil/MCAD), sample handling (frozen blood), sample handling (heat inactivation of GALT), sample contamination (bacteria)
  - Rates:
    - >90% of all initial abnormal NBS results are really unaffected
    - General FP range: 0.01 – 1.5% (varies widely from state to state, not widely reported)
      - 10 – 1500 false positives/100,000 births
  - 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
  - Causes:
    - Lab error, blood transfusion (Galactosemia), mild variants, test done too soon (maternal effects), sample storage
  - Rates:
    - Usually very low
    - Exception: Up to 1% of patients with moderate congenital adrenal hyperplasia (steroid hormone dysfunction) would have been missed in a 2005 European pilot study*

*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

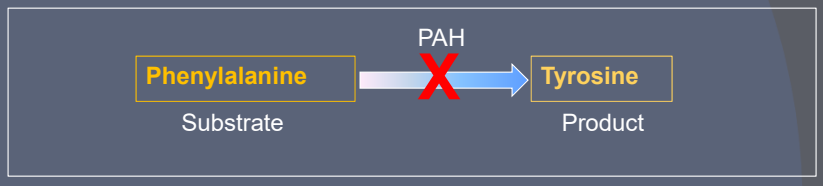
Criteria for inclusion in the core screening panel



- 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/

Historical Perspective

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

1961: Robert Guthrie pioneers a newborn screening test for PKU

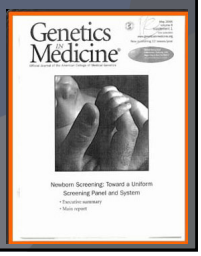
- Filter paper containing blood from newborns applied to an agar plate
- Bacteria only grow in the presence of phenylalanine
- Large colonies = PKU
- Paradigm: one test for one disorder

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 - 1980s: most states screen for ~6 conditions

A brief history of newborn screening: the era of MSMS expansion

- 1990s – early 2000s: Development and implementation of MSMS for newborn screening
  - 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

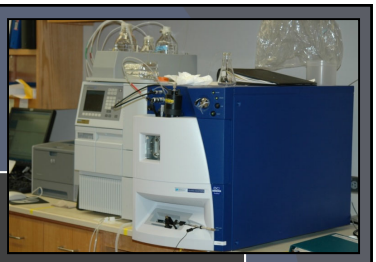


Methodology

Acylcarnitines are biomarkers for fatty acid oxidation disorders

- Deficient fatty/organic acid oxidation enzyme activity 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)

Acylcarnitines



- 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

Plasma acylcarnitine profile

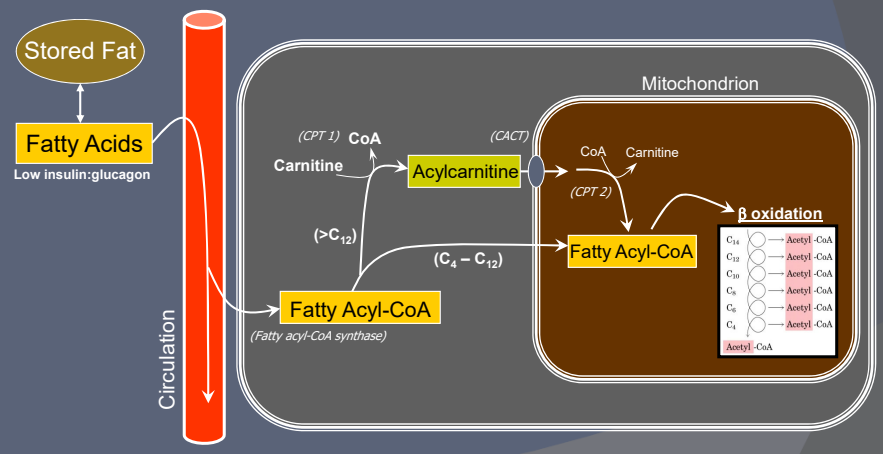
Plasma Acylcarnitine Profile	Method: ESI/MS/MS		
	µmoles/L	Normal	Note
Acetylcarnitine (C2)	20.01	9 - 34	
Propionylcarnitine (C3)	0.43	0.1 - 1.48	
Butyryl-/Isobutyrylcarnitine (C4)	0.19	0.06 - 0.64	
Tiglyl-/Methylcrotonylcarnitine (C5:1)	0.05	0 - 0.1	
Isovaleryl-/2Methylbutyrylcarnitine (C5)	0.06	0.04 - 0.33	
3-OH Butyryl/isobutyrylcarnitine (C4-OH)	0.12	0.01 - 0.17	
Hexanoylcarnitine (C6)	0.09	0.01 - 0.2	
3OH-IVA/3OH-2MeButyryl(C5-OH)	0.06	0 - 0.16	
3-OH Hexanoylcarnitine (C6-OH)	0.01	0 - 0.05	
Octenoylcarnitine (C8:1)	0.57	0.05 - 0.75	
Octanoylcarnitine (C8)	0.15	0 - 0.36	
Malonylcarnitine (C3DC)	0.07	0.01 - 0.2	
Decenoylcarnitine (C10:1)	0.14	0 - 0.49	
Decanoylcarnitine (C10)	0.14	0 - 0.48	
Methylmalonyl-/Succinylcarnitine (C4DC)	0.03	0 - 0.23	
Glutarylcarnitine (C5DC)	0.05	0 - 0.25	
Dodecenoylcarnitine (C12:1)	0.02	0 - 0.16	
Dodecanoylcarnitine (C12)	0.12	0.01 - 0.19	
Adipoyl-/Methylglutarylcarnitine (C6DC)	0.04	0 - 0.16	
3-OH Dodecanoylcarnitine (C12-OH)	0.03	0 - 0.07	
Tetradecadienoylcarnitine (C14:2)	0.04	0 - 0.13	
Tetradecenoylcarnitine (C14:1)	0.05	0 - 0.18	
Tetradecanoylcarnitine (C14)	0.04	0 - 0.17	
3-OH Tetradecenoylcarnitine (C14:1-OH)	0.02	0 - 0.13	
3-OH Tetradecanoylcarnitine (C14-OH)	0.01	0 - 0.08	
Hexadecenoylcarnitine (C16:1)	0.05	0 - 0.1	
Hexadecanoylcarnitine (C16)	0.07	0 - 0.24	
3-OH Hexadecenoylcarnitine (C16:1-OH)	0.01	0 - 0.06	
3-OH Hexadecanoylcarnitine (C16-OH)	0.01	0 - 0.07	
Linoleoylcarnitine (C18:2)	0.04	0.02 - 0.16	
Oleoylcarnitine (C18:1)	0.09	0.03 - 0.23	
Stearoylcarnitine (C18)	0.05	0.01 - 0.14	
3-OH Linoleoylcarnitine (C18:2-OH)	0.02	0 - 0.05	
3-OH Oleoylcarnitine (C18:1-OH)	0.02	0 - 0.06	

ND: analyte below limits of detection

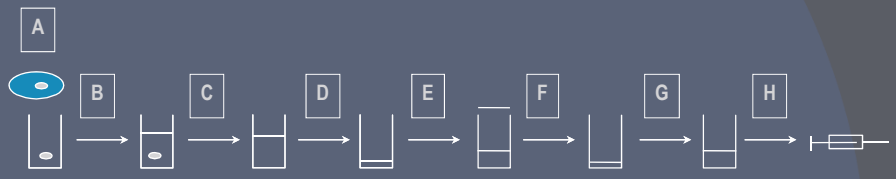
Acylcarnitines, continued

- Sample requirements
  - Plasma (≥1 cc)
    - 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)

Overview of fatty acid oxidation



Blood Spot Sample Preparation

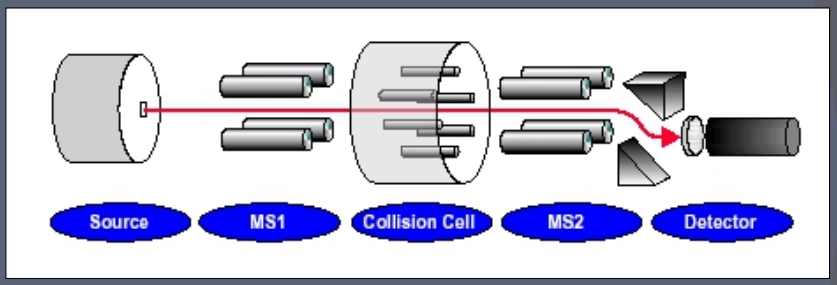


A. Punch out one spot from Guthrie card (typically 3/16" or 3mm).
B. Add 100 µL MeOH (with internal standards) and extact for 30 minutes
C. Transfer supernatant into second plate.
D. Evaporate to dryness under nitrogen with mild (40°C) heating.
E. Add 100 µL 3 N Butanolic HCl to each sample and heat 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

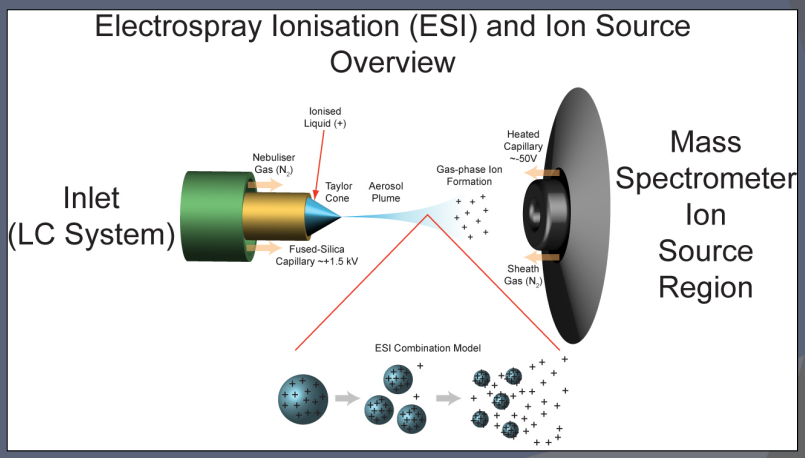
Waters Quattro Micro LC-MSMS

- Triple quadrupole mass spectrometer with electrospray ionization

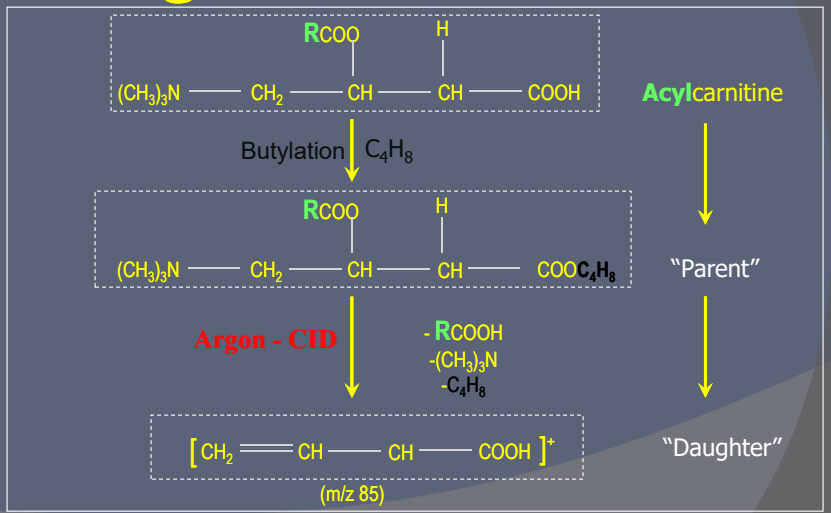
Schematic of a triple quadrupole tandem mass spectrometer



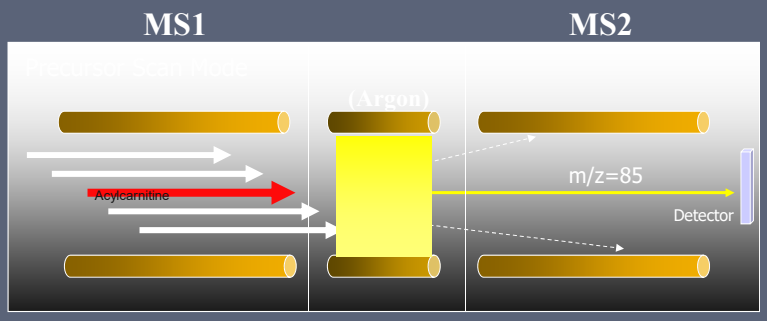
Electrospray ionization



Acylcarnitines: derivatization and fragmentation



Analysis of plasma acylcarnitines using precursor scanning ("parents of 85")

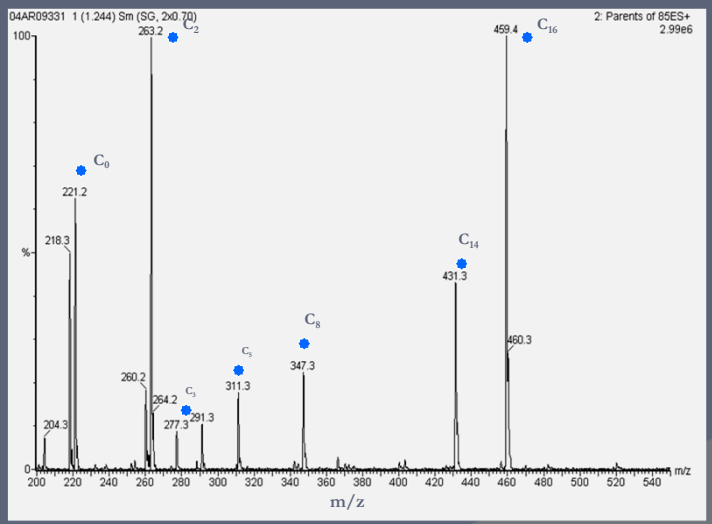


Scanning:
-sequential passage of all masses

CID

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

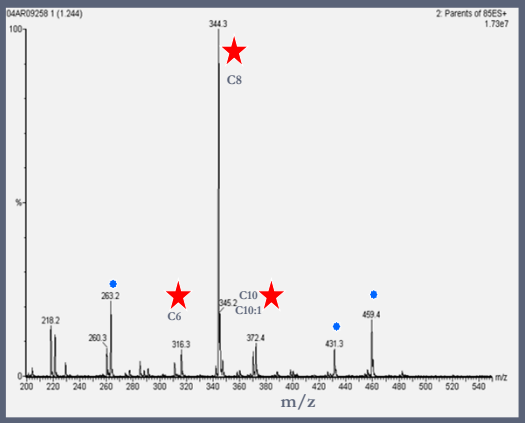
Plasma acylcarnitine profile



Normal profile

= internal standard peak

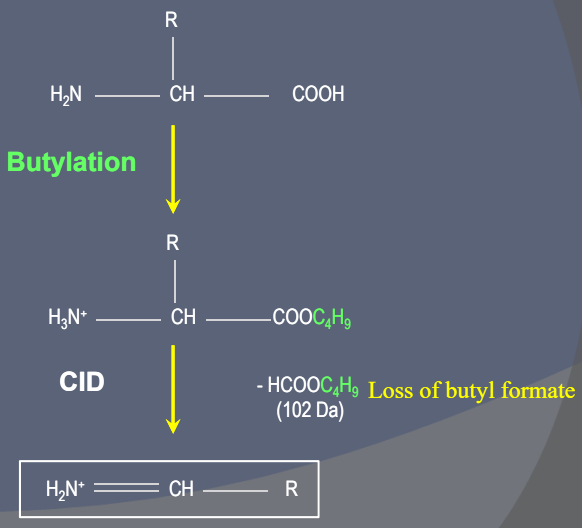
Abnormal acylcarnitine profile



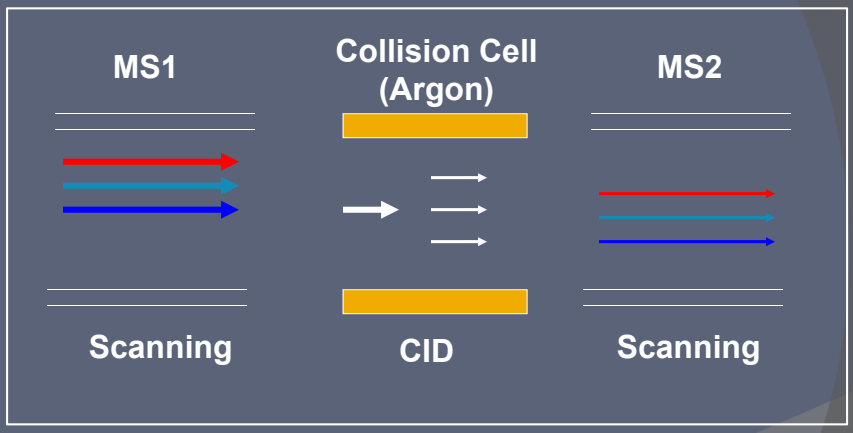
MCAD

- Medium Chain Acyl-CoA Dehydrogenase (MCAD) deficiency
- Most common defect of mitochondrial FAO (1:15,000)
- Lethargy, seizures, hypoketotic hypoglycemia, sudden death
- Diagnosis allows for treatment (avoidance of fasting)
  - Clinical utility

Neutral and acidic amino acids: derivatization and fragmentation

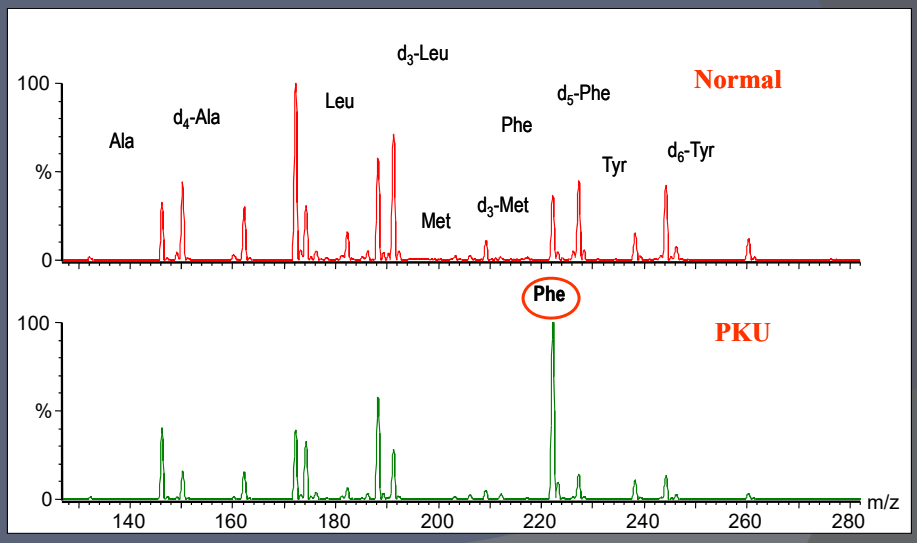


Neutral Loss Scan for Amino Acids

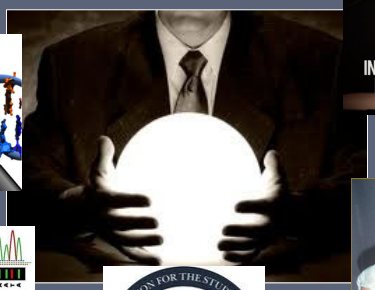


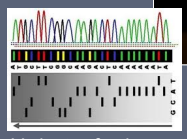
- Loss of 119 Da for basic amino acids
- Loss of 102 Da for acidic and neutral amino acids

Phenylketonuria (PKU)

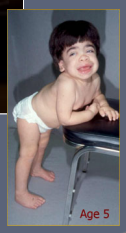


The Future of Newborn Screening





Variants of unknown significance



Genzyme

Google images

Where do we go from here?

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

Altering the paradigm: 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

Thank You!

UAB DEPARTMENT OF GENETICS