Metabolomics and You

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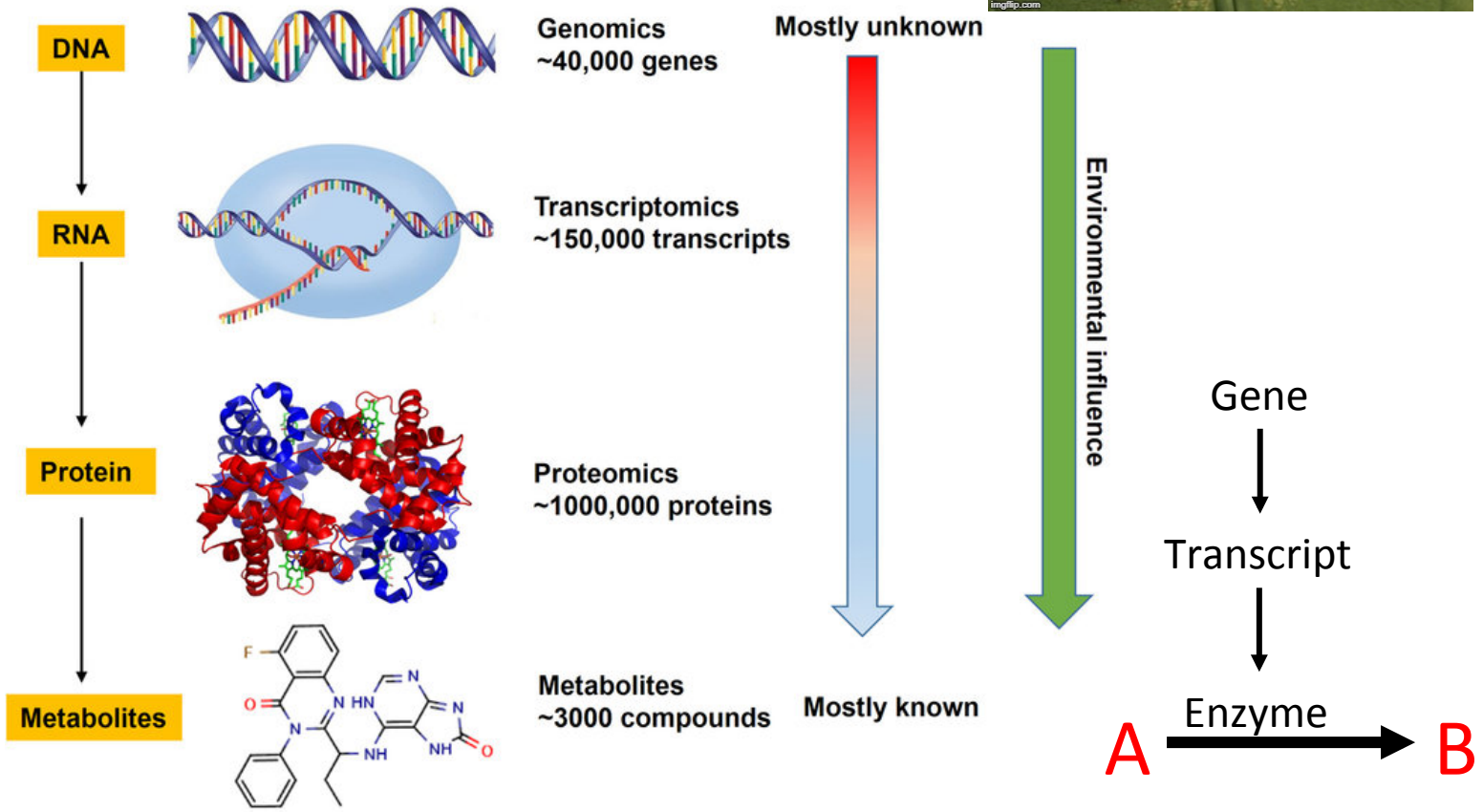
Objectives

- Define “metabolomics”.
- Describe how metabolomics is used currently in research. In the clinical setting?
- Identify potential uses for metabolomic analyses in newborn screening.

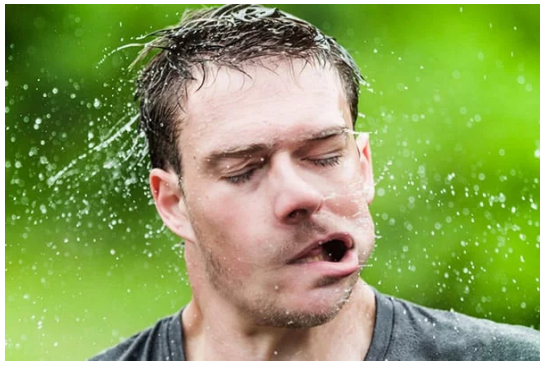
What is Metabolomics?

- "-Omics": The collective technologies used to explore the roles, relationships, and actions of the various types of molecules that make up the cells of an organism
  - Genomics/Epigenomics
  - Transcriptomics
  - Proteomics
  - Metabolomics

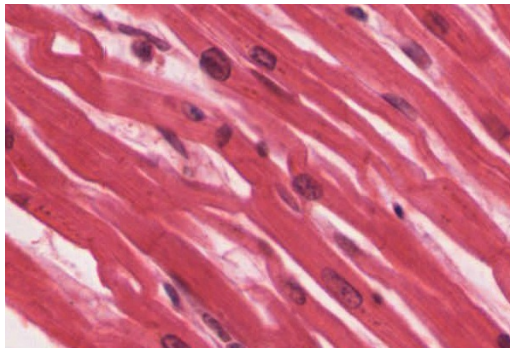
  - Phenomics
  - Lipidomics
  - Glycomics
  - Foodomics
  - Inflammomics
  - Beeromics



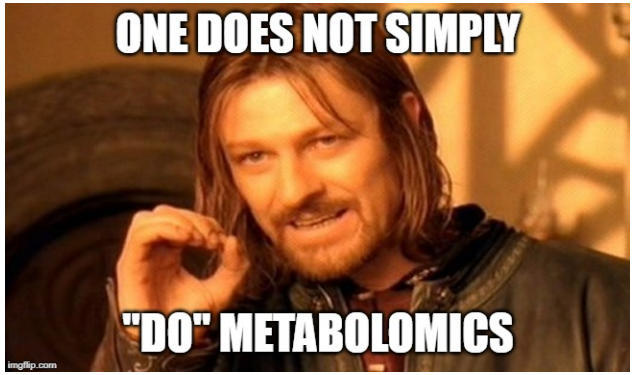
What is Metabolomics?



- Comprehensive analysis of metabolites (A, B, …Z) in a biological sample
  - Tissue sample/biopsy
  - Cell culture
  - Blood
  - Urine
  - CSF
  - Breath
  - Sweat



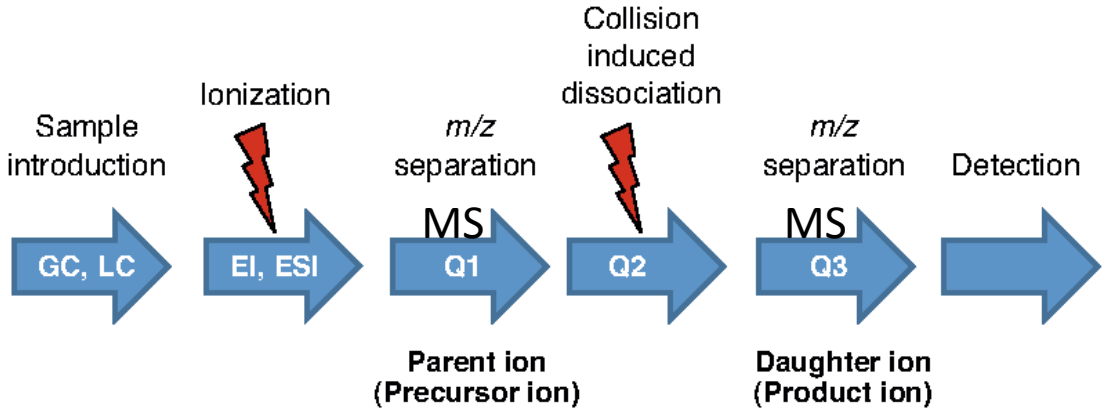
- Typically “small molecules” (amino acids, organic acids, nucleic acids, acylcarnitines, fatty acids, biogenic amines, etc.)
- May look at lipids (lipidomics), large molecules (GAGs), etc.
- Any species or model

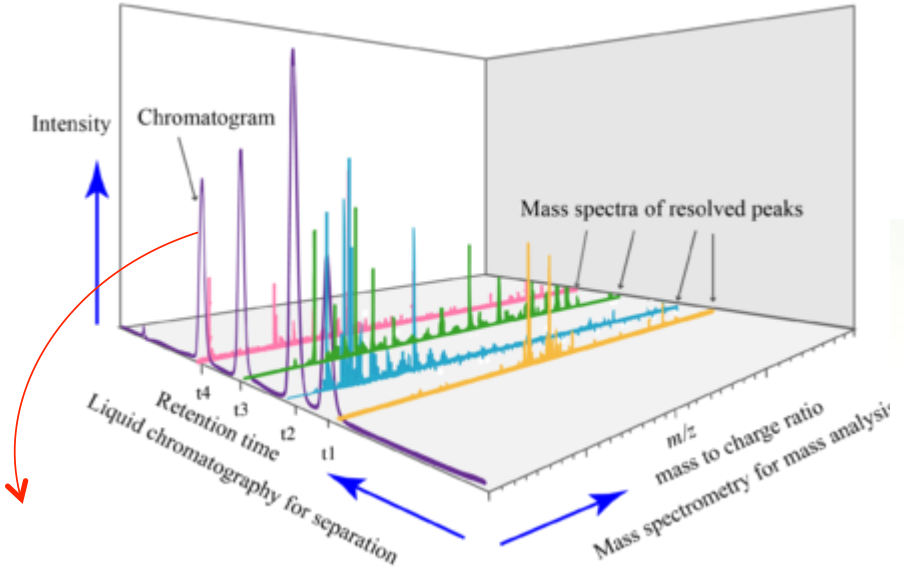


- Characterizes the **substrates** and **products** of metabolism in the sample/organism as a **functional phenotype**

Metabolomics Methods

- MS/MS or NMR spectrometry
- May use Gas (GC-MS) or liquid (LC-MS) chromatography to separate molecules
- 1st MS: peak intensity and separation, sample "fingerprint"
- 2nd MS: molecule "fingerprint" for ID
- Peak intensity (relative to internal standards) allow for quantification
  - relative abundance
  - actual units (eg micromolar)





arginine

= 375 "units"

Metabolomics Methods: Targeted v Untargeted Analyses

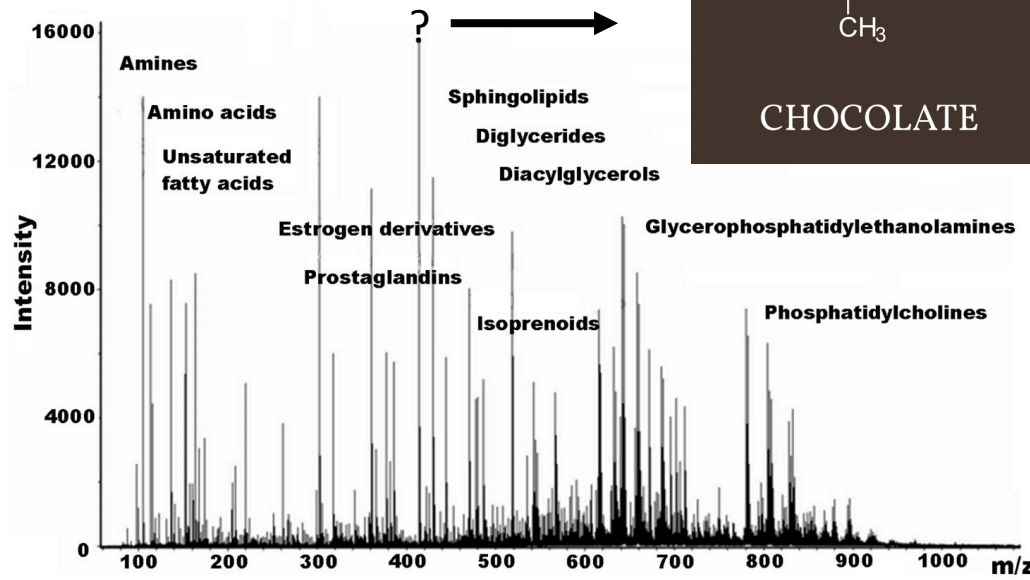
Metabolomics

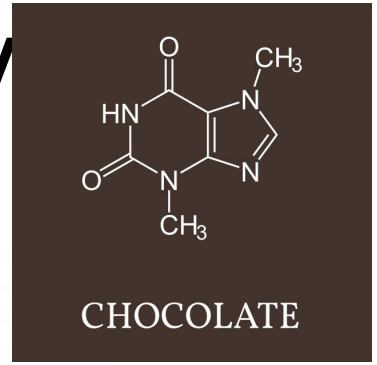
Discovery

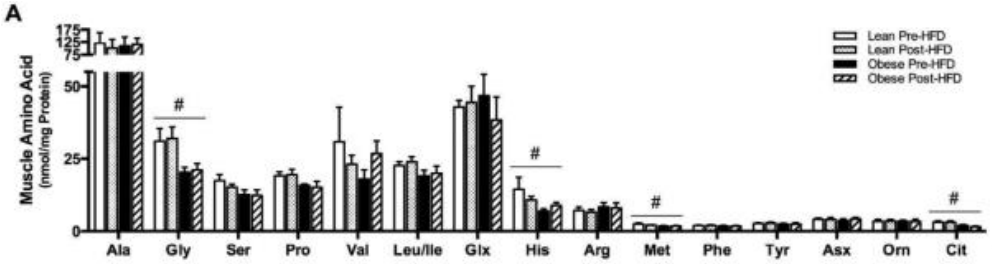
Global Untargeted
- Hypothesis-generating
- Global/Comprehensive analysis
- Correlated to databases/libraries
- Qualitative identification
- Relative quantification

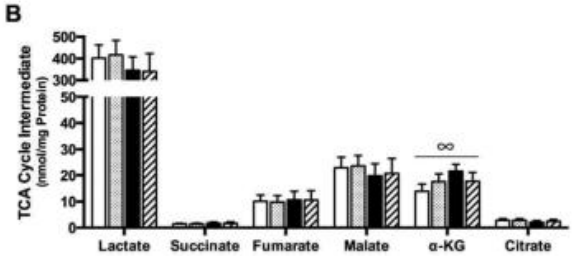
Validation/Quantification

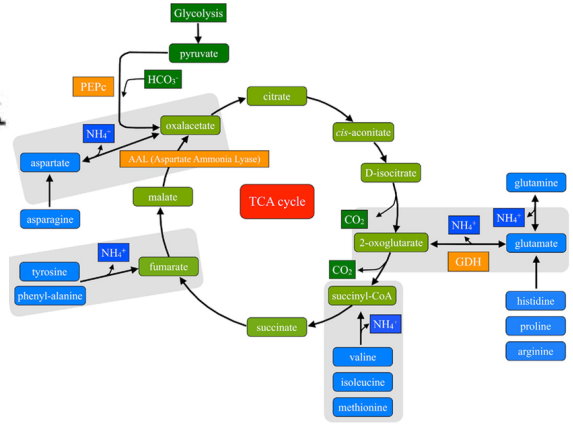
Targeted
- Hypothesis-driven
- Subset analysis
- Correlated to reference standards
- Identification already known
- Absolute quantification

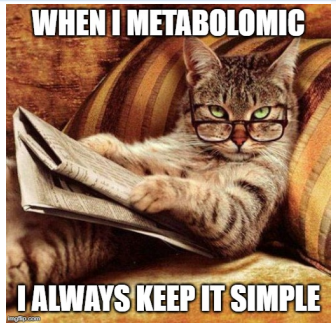






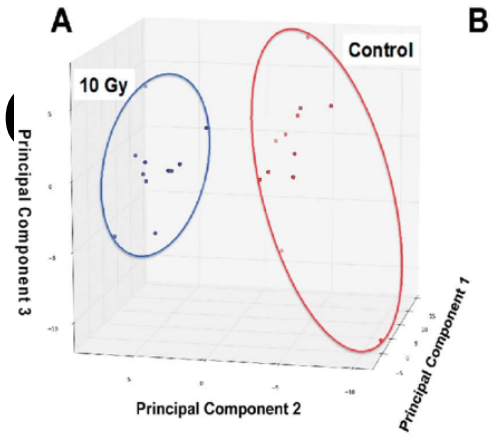


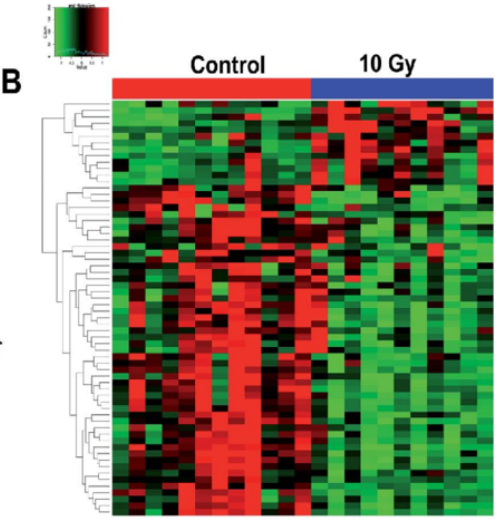


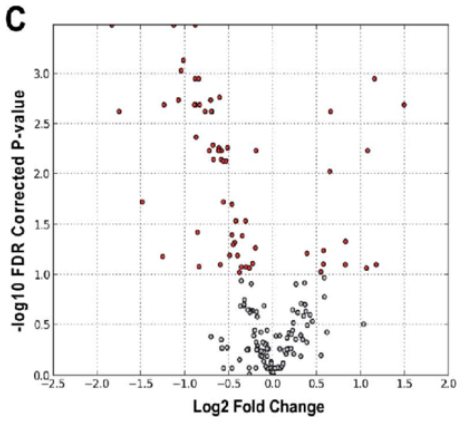


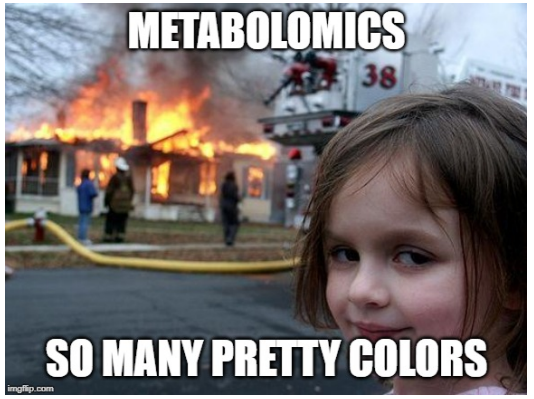
Metabolomics Analysis

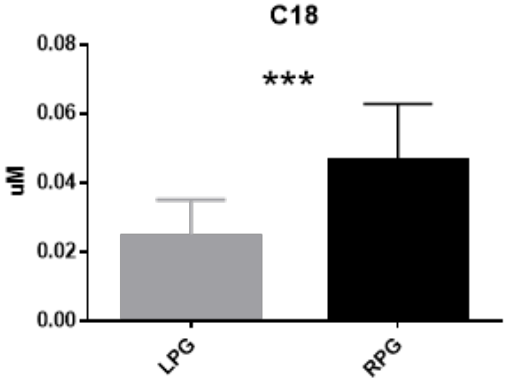
- Obtained relative or absolute values for X number of analytes
- Use GraphPad, Excel, R software to analyze values:
  - Compare groups and see which analytes are significantly different
    - Student T-test, Chi-Square
  - Test analyte concentration correlations with a continuous phenotypic variable
    - Pearson's Correlation
  - Advanced analysis (random forest, volcano plot, principle component analysis, heat map, hierarchical clustering)

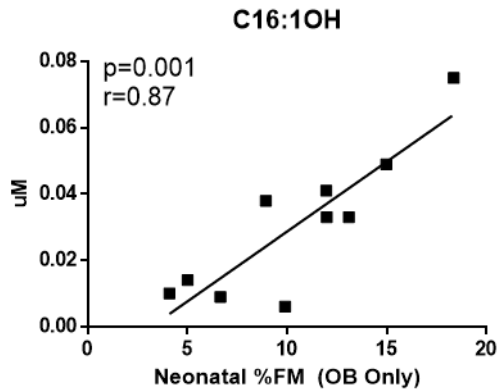




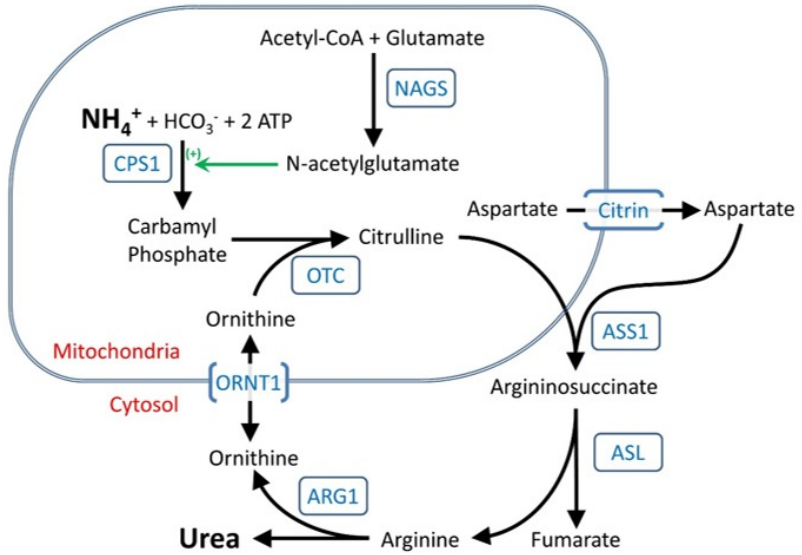








Map Results onto Pathways



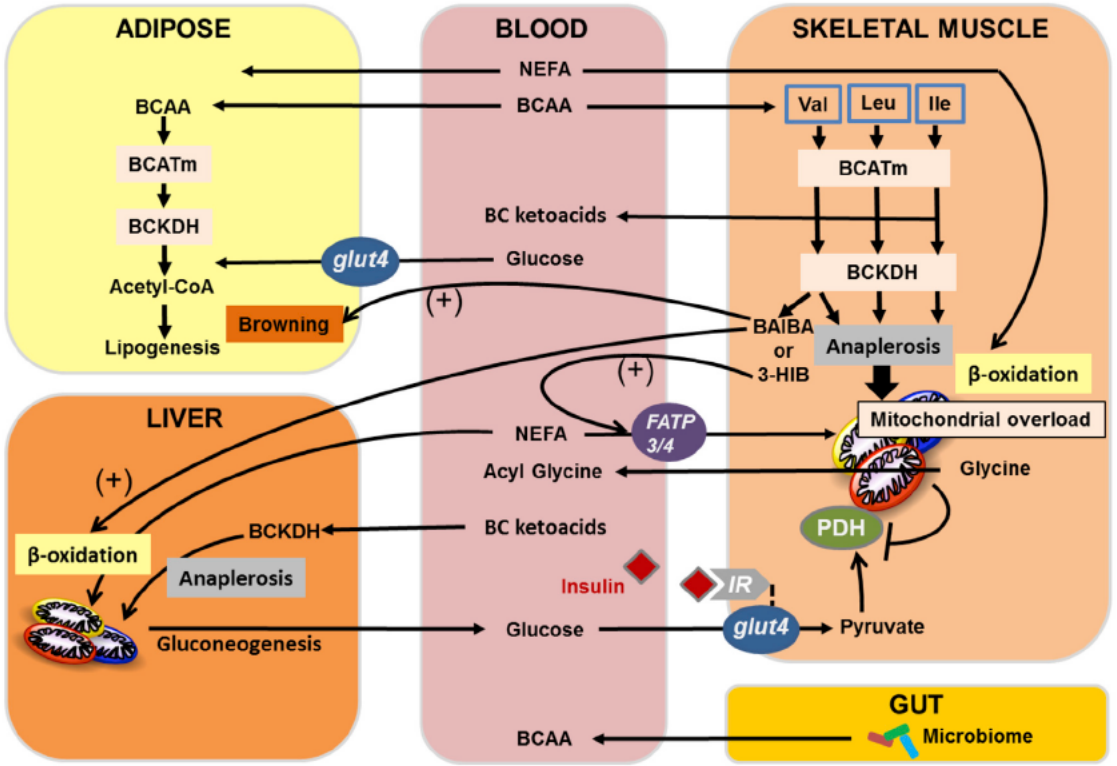
Test for individual biomarkers
or
Test for pathway enrichment
KEGG
HMDB
MetaboAnalyst

Goals:

1) **Biomarkers to predict disease (onset, response to Rx, progression, severity)**
2) **Pathway enrichment to understand mechanism/physiology/targets**

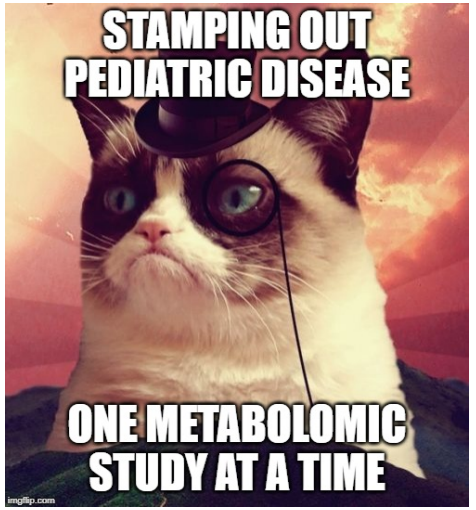
Metabolomics in Research

- Obesity and Type 2 Diabetes
- Non-alcoholic Fatty Liver Disease
- Myocardial Infarction and Cardiovascular Disease
- Asthma
- Cancer(s)
- Chronic Fatigue Syndrome
- Gulf War Syndrome

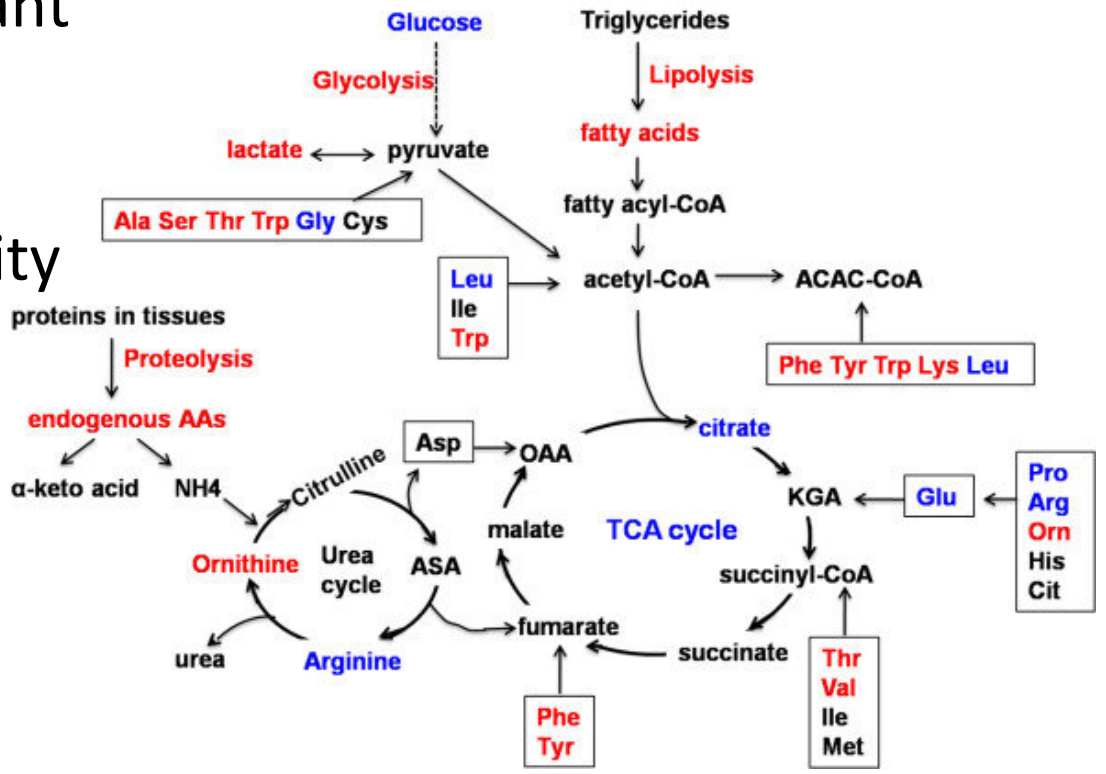


Pathway enrichment in T2DM

Metabolomics in Pediatrics



- Hypoxic ischemic encephalopathy
- Persistent ductus arteriosus
- Respiratory distress syndrome and surfactant therapy
- Cytomegalovirus infection
- Intrauterine Growth Restriction/ Prematurity
- Fetal programming in obesity/gestational diabetes
- Nephropathy
- Polycystic Ovary Syndrome



Pathway enrichment in PCOS

Metabolomics in Inherited Metabolic Disease

- Biochemical Phenotyping:
  - Fatty Acid Oxidation Disorders
  - Urea Cycle Disorders
  - Pyruvate Dehydrogenase Deficiency
  - Peroxisomal disorders
  - Neuronal Ceroid Lipofuscinosis
  - GABA-transaminase deficiency
- Novel biomarkers
- Assessing treatment efficacy
- **Functional verification of genomic variants**
- Disease discovery/diagnosis
  - David Koeller/UDN, SIMD 2019: Not yet ready for prime time
- We use clinically in rare occassions

Mol Genet Metab. 2019 Jul 16. pii: S1096-7192(19)30414-7. doi: 10.1016/j.ymgme.2019.07.007. [Epub ahead of print]

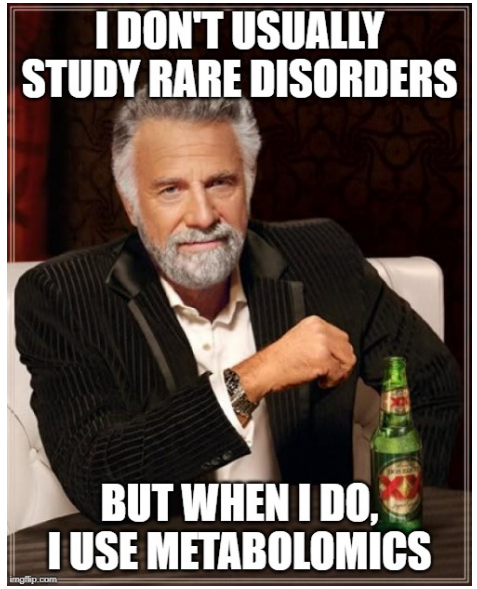
Metabolite flux: A dynamic concept for inherited metabolic disorders as complex traits.

McCabe ERB[1].

J Inherit Metab Dis. 2018 Sep;41(5):753-756. doi: 10.1007/s10545-018-0209-9. Epub 2018 Jun 6.

The phenotype of adult versus pediatric patients with inborn errors of metabolism.

Saudubray JM[1], Mochel F[2,3,4,5].



J Inherit Metab Dis. 2018 May;41(3):329-336. doi: 10.1007/s10545-018-0137-8. Epub 2018 Apr 16.

The role of the Human Metabolome Database in inborn errors of metabolism.

Mandal R[1], Chamot D[1], Wishart DS[2,3,4].

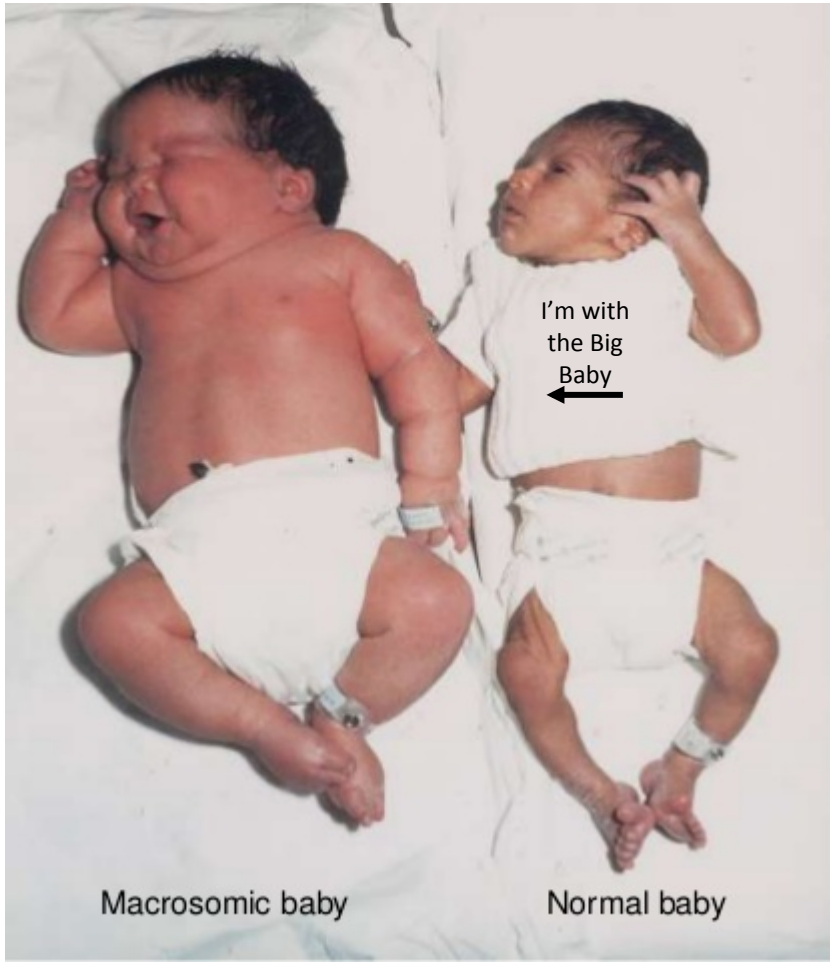
Metabolomics in Newborn Screening

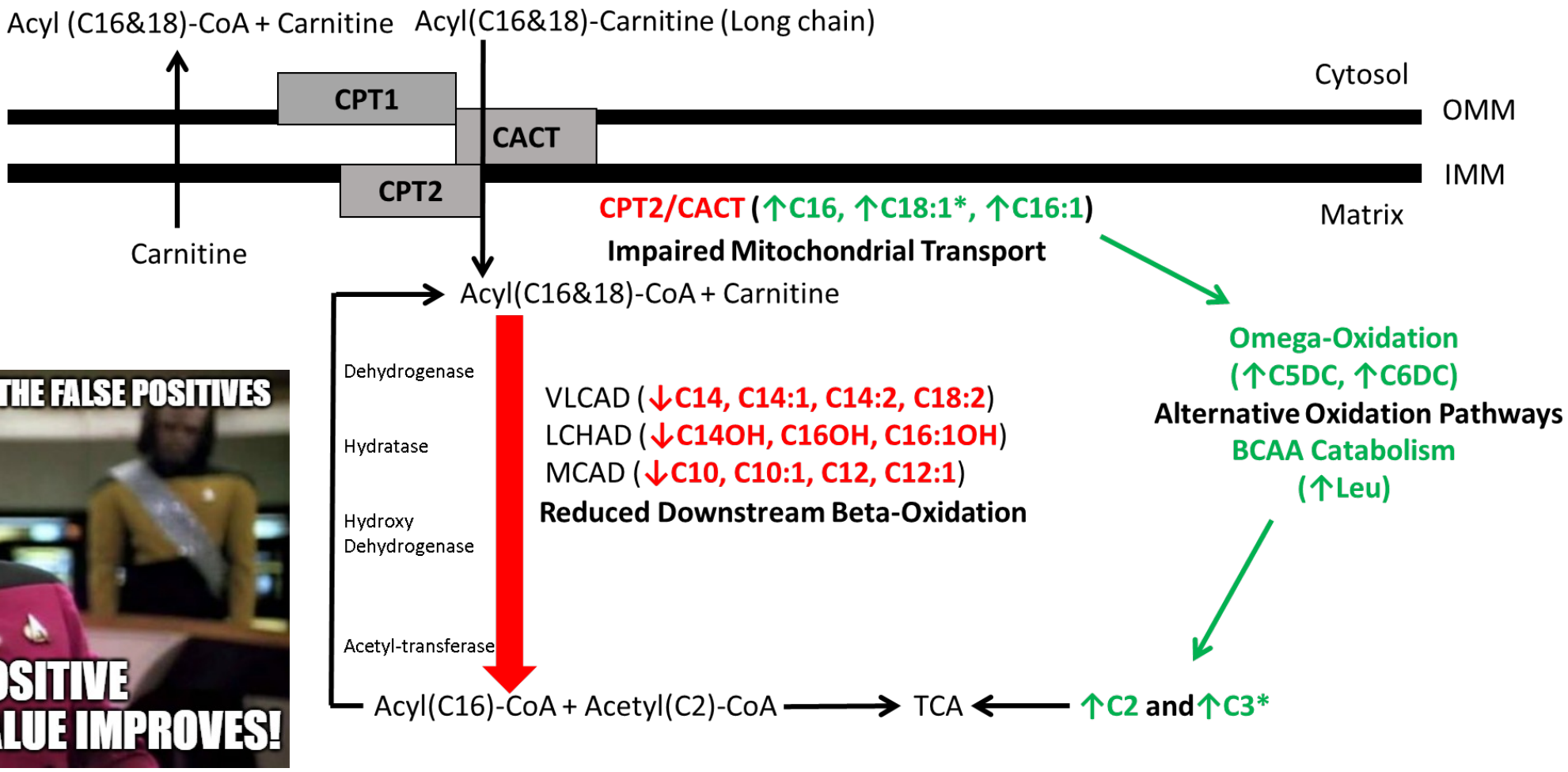
- Isn't the MS/MS based NBS **already** a targeted metabolomic analysis?
- Potential utility:
  - Better positive predictive value/ avoid false positives
  - More specific (**personalized**) cut-offs (CLIR)
  - Rapid second tier testing
  - Personalized medicine:
    - Individualized lifetime medicine
    - Tailored neonatal/pediatric care
    - Better understanding of common dz
    - Early prediction of pediatric dz
- Example…

Obligatory NBS pic

Wright and Baker, in review JCEM

- Colorado experience with macrosomia as an interfering factor on NBS
- Macrosomic vs Normal Weight based on DBS card
  - Jan 1, 2016 – Dec 31, 2018
  - 24-72hrs of life
  - 2500-3999 grams = NW
  - 4000-8000 grams = Macro
  - No transfusions
  - Only non-identifiable information on the NBS card as archived by CDPHE
- Total n=~140,000 samples





Disorder	Analyte	Cut-Off (uM)	2500-3999 (n=131,896)	4000-8000 (n=7,806)	OR	CI 95%	p-value
MMA/PA	C3	≥5	0.67%	1.59%	2.4	2.0 to 2.9	<0.0001
GA1	C5DC	≥0.5	0.05%	0.02%	NS	NS	0.59
CPT2/CACT	C16	≥8	0.05%	0.06%	NS	NS	0.92
CPT2/CACT	C18:1	≥2.5	0.89%	1.58%	1.8	1.5 to 2.1	<0.0001
MSUD	Leu	≥270	0.46%	0.56%	NS	NS	0.20

Higher potential false positives for C3 (Methylmalonic and Propionic Acidemia) in Macrosomic vs NW newborns in CO

New insight into pathophysiology in macrosomia

Conclusions

- Metabolomics is:
  - The study of small molecules to determine phenotypic/functional differences between groups
  - Already using in NBS (since 2000's)
  - Prevalent in research for adult, pediatric, and metabolic disease
  - Not quite ready to make a large clinical impact
- Metabolomics in relation to IEM and the NBS *could eventually*:
  - Improve quality and precision of results
  - Better characterize pathophysiology
  - Create a means of precision medicine in rare (and common) disease detection and management