

EMORY UNIVERSITY  
DEPARTMENT OF MEDICINE  
Clinical Biomarkers  
Laboratory

July 25, 2013

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UAB Metabolomics  
Workshop

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## Metabolomics Pathway Analysis

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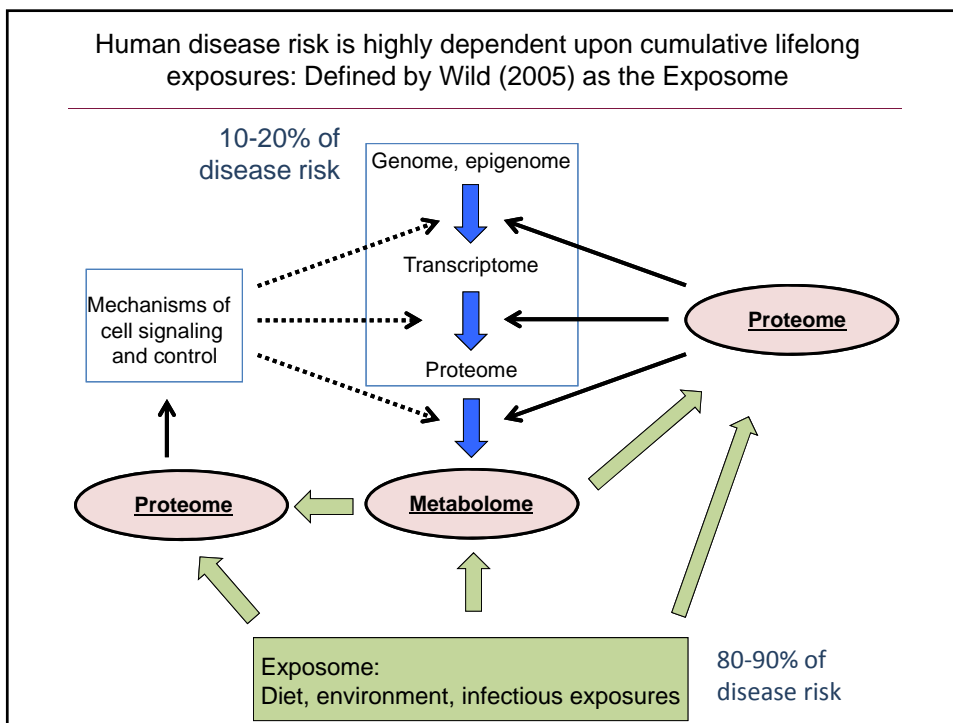
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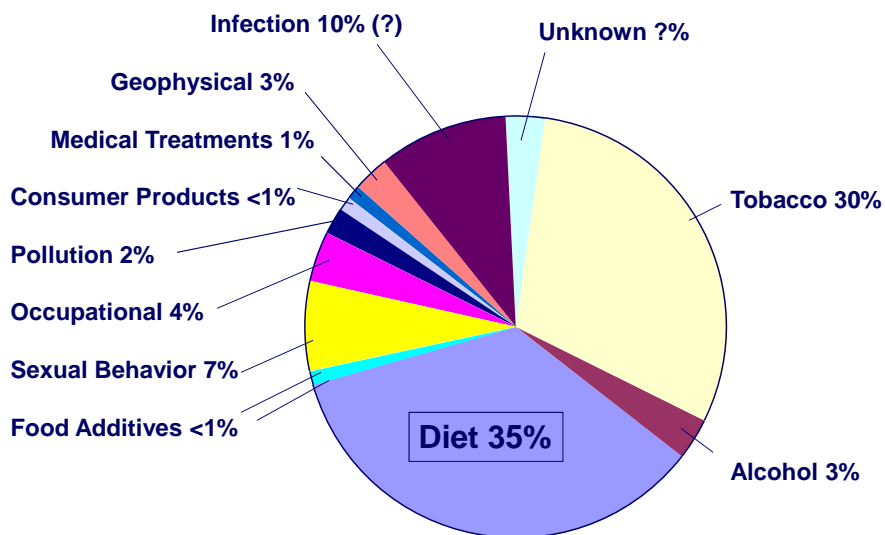
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No financial COI to disclose

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## Proportion of cancer deaths attributed to various environmental factors (Doll and Peto)



## Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study

Karen Barnett, Stewart W Mercer, Michal Norbury, Graham Watt, Sally Wyke, Bruce Guthrie

### Summary

**Background** Long-term disorders are the main challenge facing health-care systems worldwide, but health systems are largely configured for individual diseases rather than multimorbidity. We examined the distribution of multimorbidity, and of comorbidity of physical and mental health disorders, in relation to age and socioeconomic deprivation.

Lancet 2012; 380: 37-43  
 Published Online  
 May 10, 2012  
 DOI: 10.1016/S0140-6736(12)60240-2

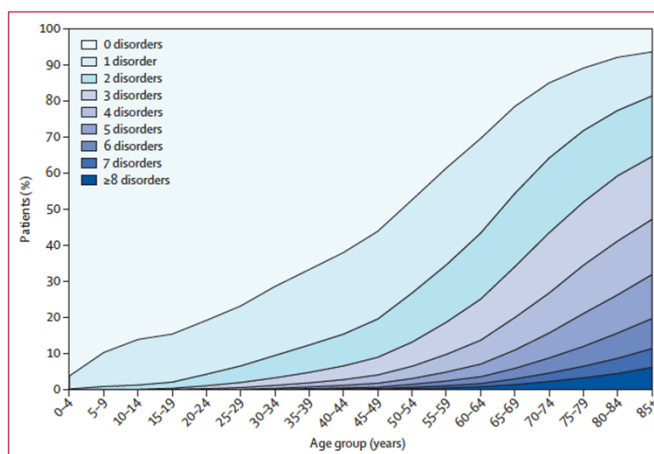


Figure 1: Number of chronic disorders by age-group

## Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study



Karen Barnett, Stewart W Mercer, Michael Norbury, Graham Watt, Sally Wyke, Bruce Guthrie

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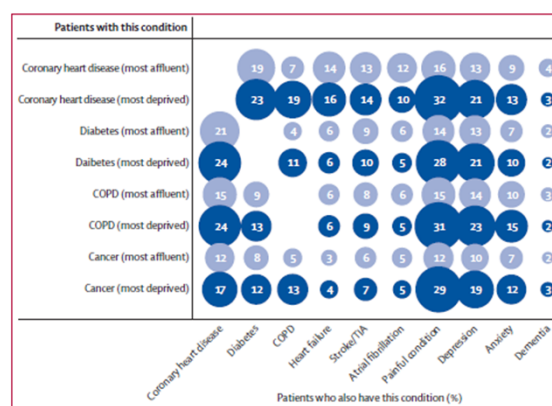


Figure 4: Selected comorbidities in people with four common, important disorders in the most affluent and most deprived deciles  
COPD=chronic obstructive pulmonary disease, TIA=transient ischaemic attack.

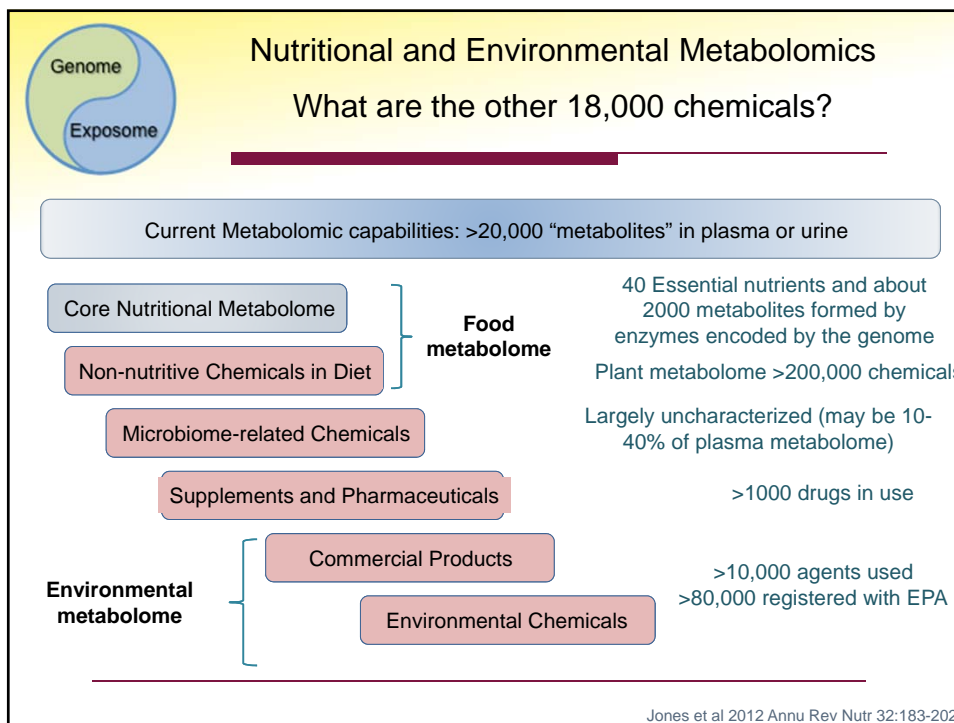
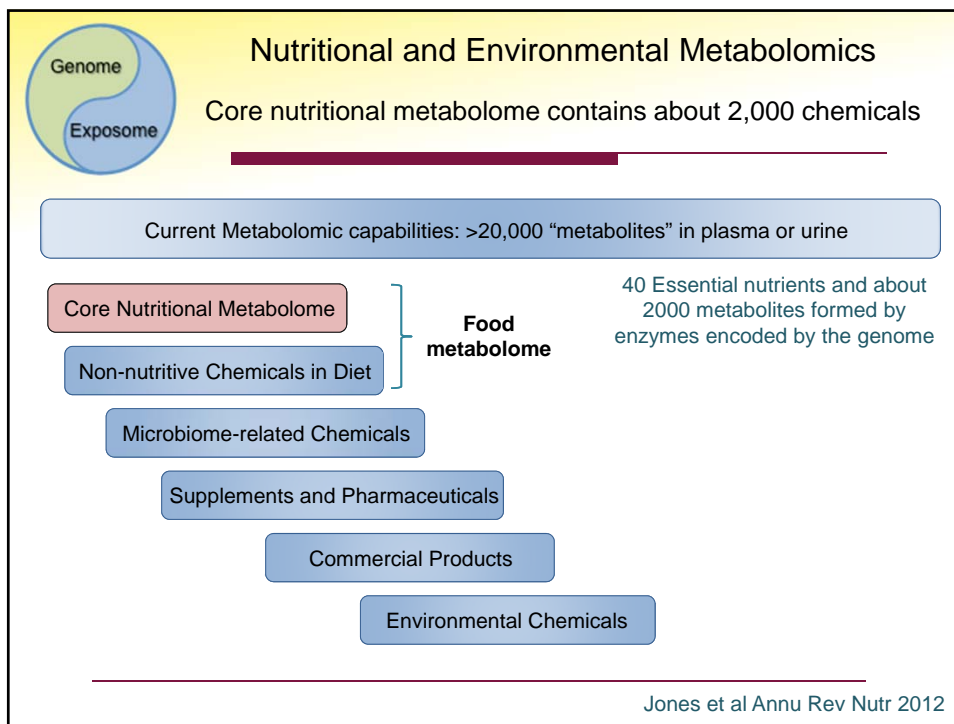
## Alternate Workflows


### Targeted Metabolomics

Select analytic target to test hypothesis  
↓  
Select and test analytic method  
↓  
Perform power calculation; design experiment  
↓  
Conduct experiment  
↓  
Analyze samples and perform statistical analysis

### High-resolution metabolomics

Pose scientific question (with or without hypothesis)  
↓  
Select relevant samples  
↓  
Analyze samples by high-resolution MS with advanced data extraction algorithms  
↓  
Use bioinformatic methods and database tools to obtain significant metabolites and pathways  
↓  
Perform MS/MS and co-elution studies to verify metabolites





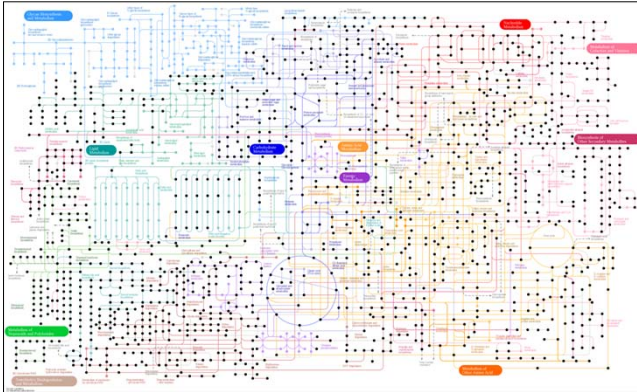
*High-resolution metabolomics is becoming practical for routine healthcare*

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**Resolution approaches that of genomics: >20,000 metabolites**

Simple 1-step sample processing can be done anywhere  
 Relatively rugged instruments can be used in hospitals and larger clinics  
 10 to 60 min run time; currently \$50 to \$125/sample, cost decreasing

In conjunction with an online health surveillance and forecasting system, metabolomics analysis could have real-time use in clinical practice



Jones et al 2012 Annu Rev Nutr 32:183-202

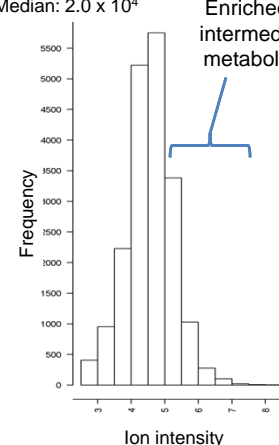
### High-resolution metabolomics data for 174 serum samples

**Improved data extraction over most approaches:** 34,768 ions, triplicate analyses

Summary for C18: 19,383 ions Range of detection over 5 orders of magnitude of intensity

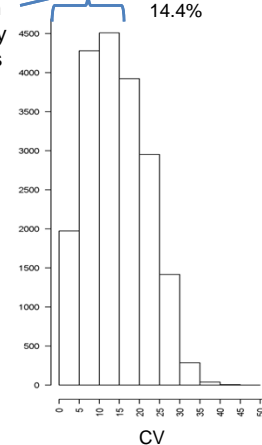
With triplicate analyses, CV is obtained for each metabolite in each sample:  
 6,247 had median CV < 10%      Mean intensity of ions with CV <10%:  $3.0 \times 10^5$

**Intensity**  
 Mean:  $1.2 \times 10^5$   
 Median:  $2.0 \times 10^4$



Ion intensity

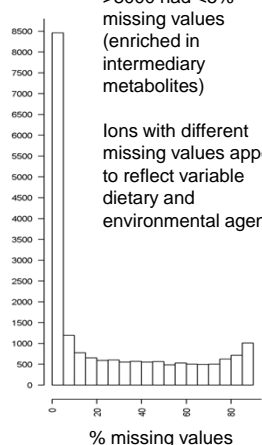
**CV**  
 Median: 14.4%



CV

**Missing values**  
 >8000 had <5% missing values (enriched in intermediary metabolites)

Ions with different missing values appear to reflect variable dietary and environmental agents

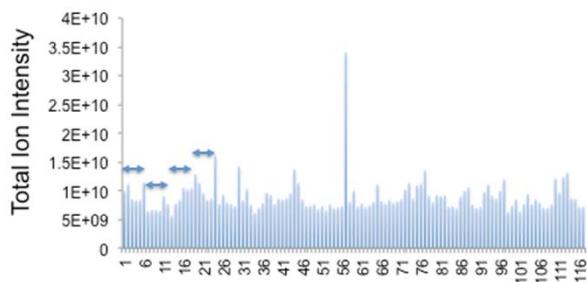
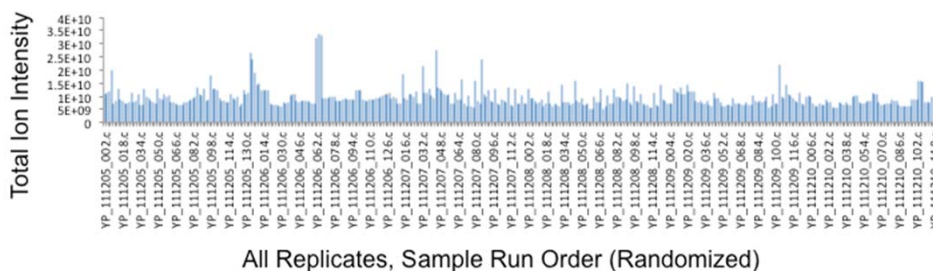


% missing values

Sample analysis (C18) shows that there is consistent LCMS system response during weeklong analysis period.

Triplicate analyses are reproducible


Random high response probably represents a residual problem with sample processing or inconsistencies in complex system (sample, autosampler and injector components, multiple valves, electrospray inhomogeneities or electromagnetic properties of ion transfer tube)



Averages of replicates includes random variation of individual high values but otherwise shows that individuals have relatively consistent total signal.

It is not clear whether individuals have differences in total signal due to amount of total metabolites—we concluded in our earlier NMR studies of SAA insufficiency that this occurred, perhaps due to differences in amount of albumin

An alternative possibility is that there are specific chemicals in some individuals that have global effects on ionization. This could be NaCl content, phosphate, sulfate, total lipid or other high-abundance chemical. Expectation is that it would be principally impacting the initial (salt) washthrough



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**Key components of pathway analysis**

**Statistical testing: FDR**

**Metabolite-metabolite Correlation Analyses**

**Online Databases/Resources**

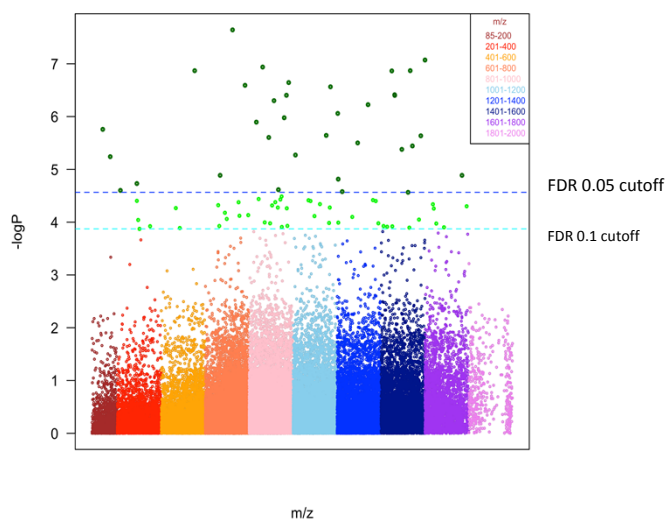
**Cross-platform Studies**

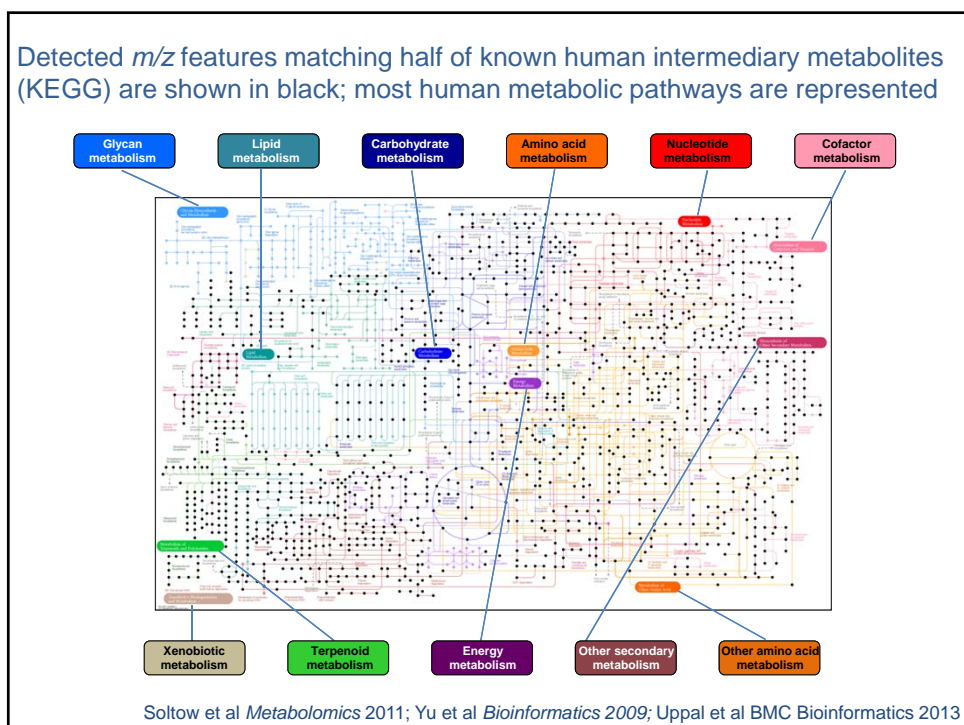
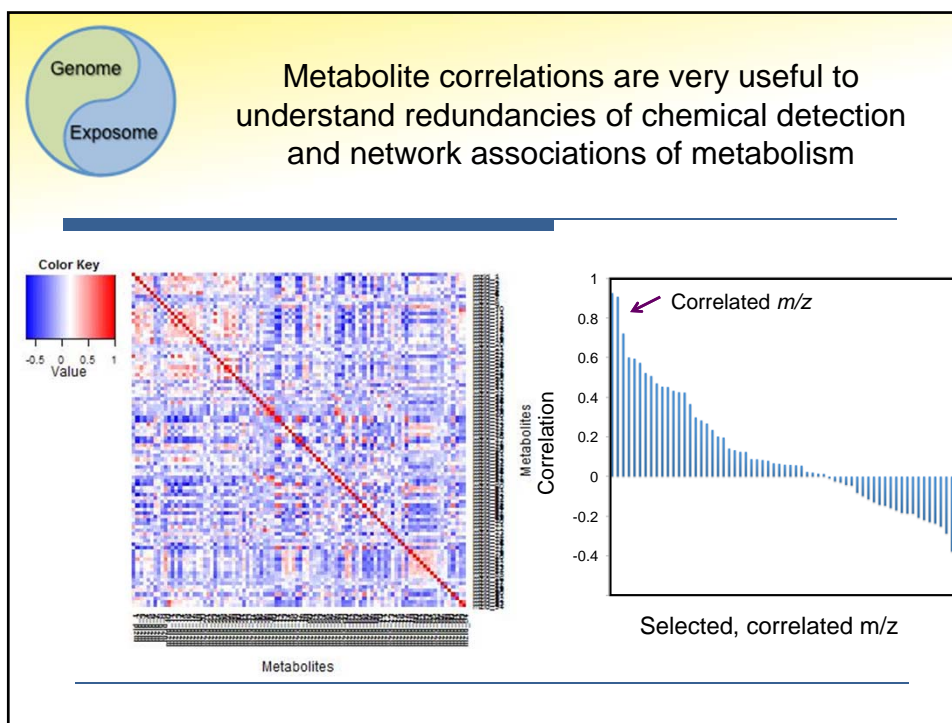


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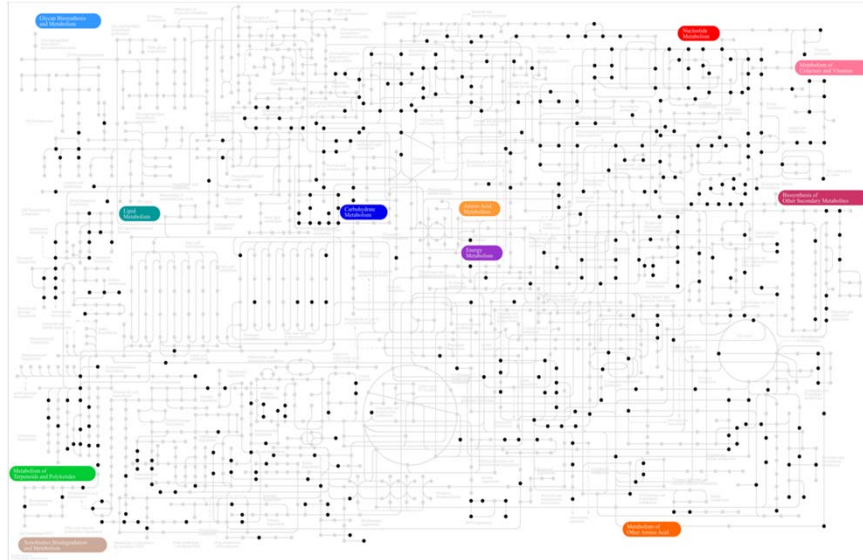
**Manhattan plot:** Y axis represents the negative  $\log_{10}$  of p-value (higher is better) and the x-axis represents the measured m/z



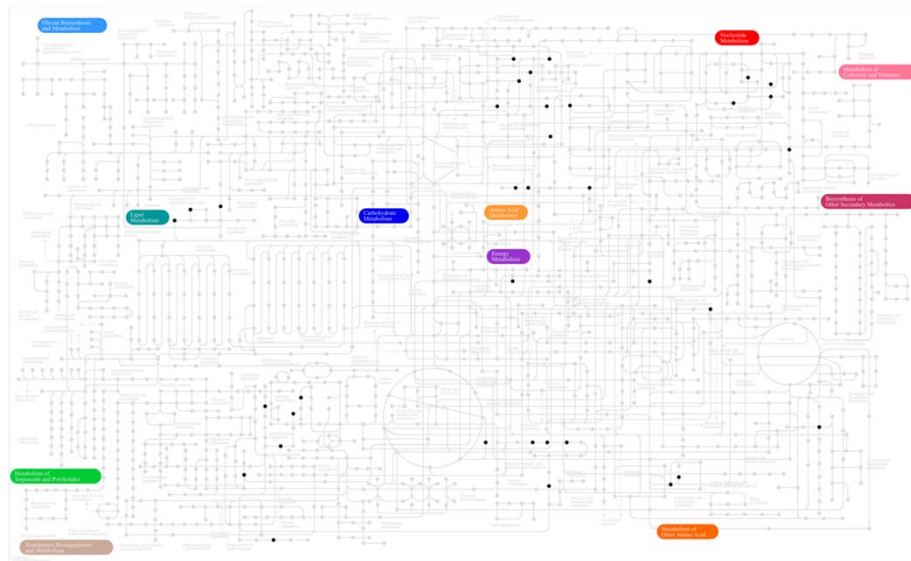


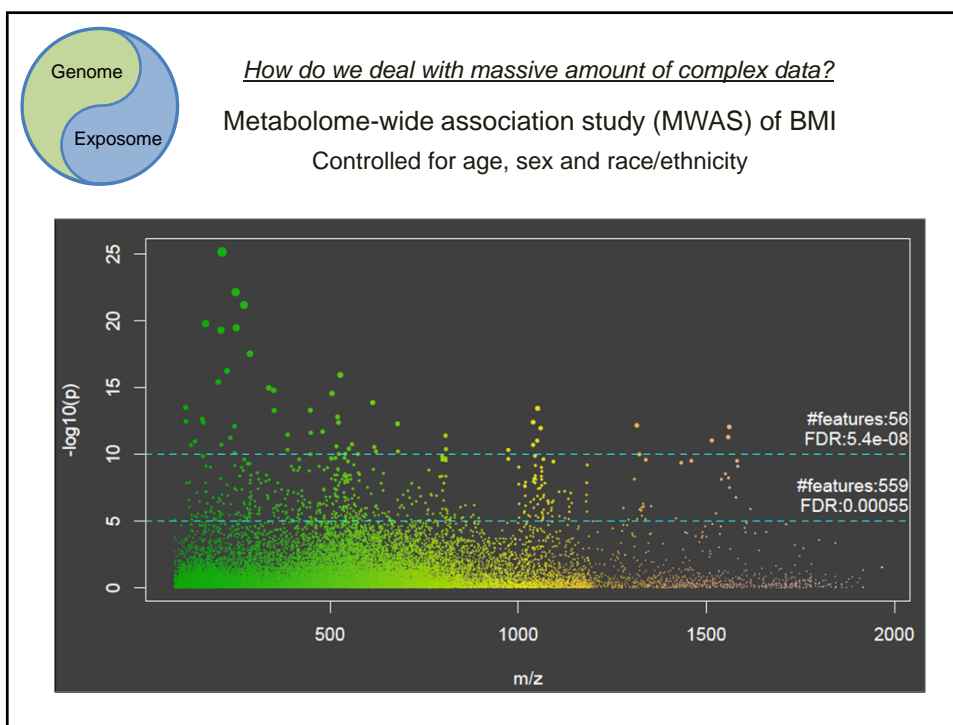
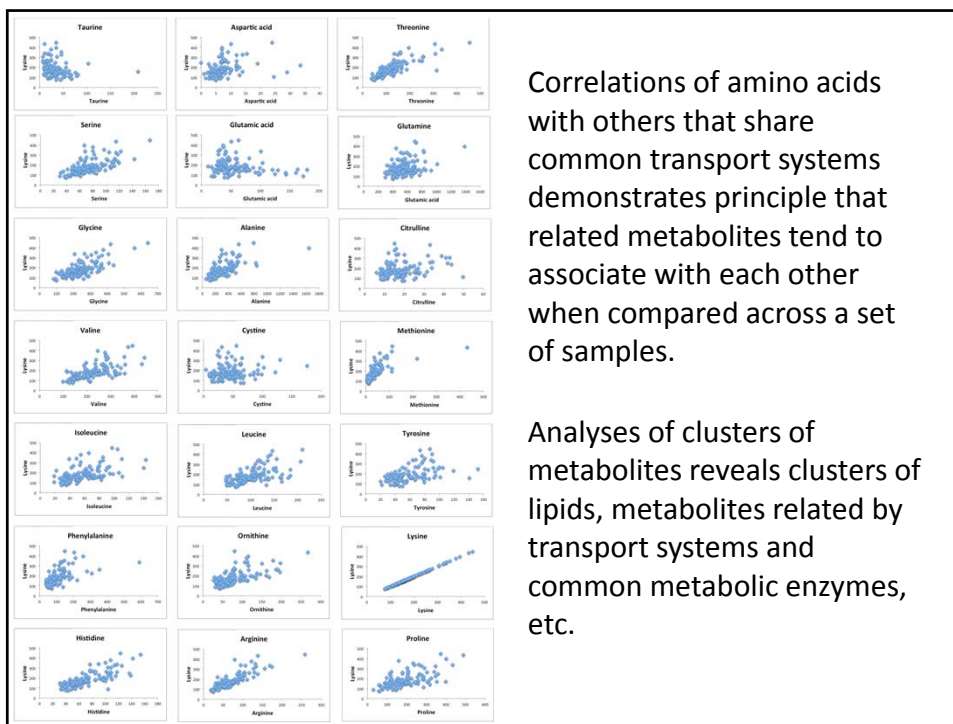


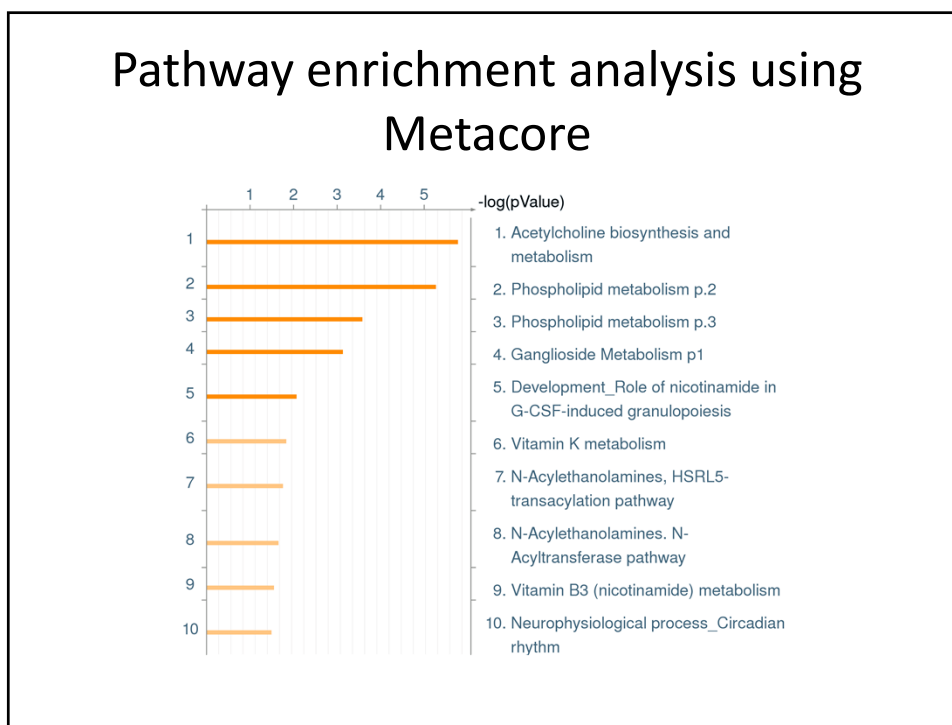
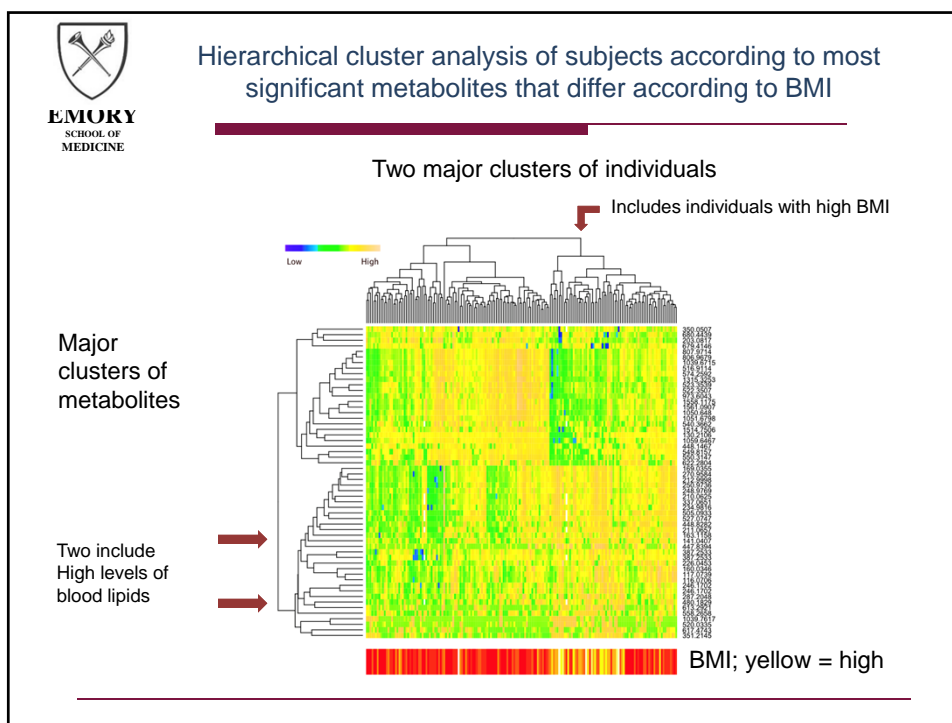
### Pathway Analysis of 400 matched $m/z$ significantly different between healthy controls and patients



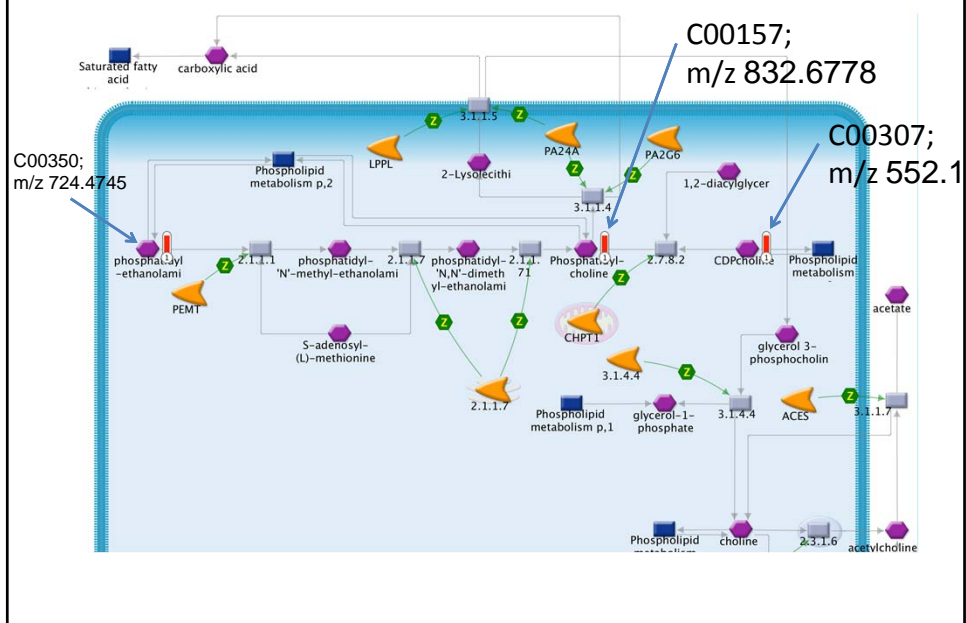
### Pathway Analysis of 35 features contributing to PCR correlation to disease score





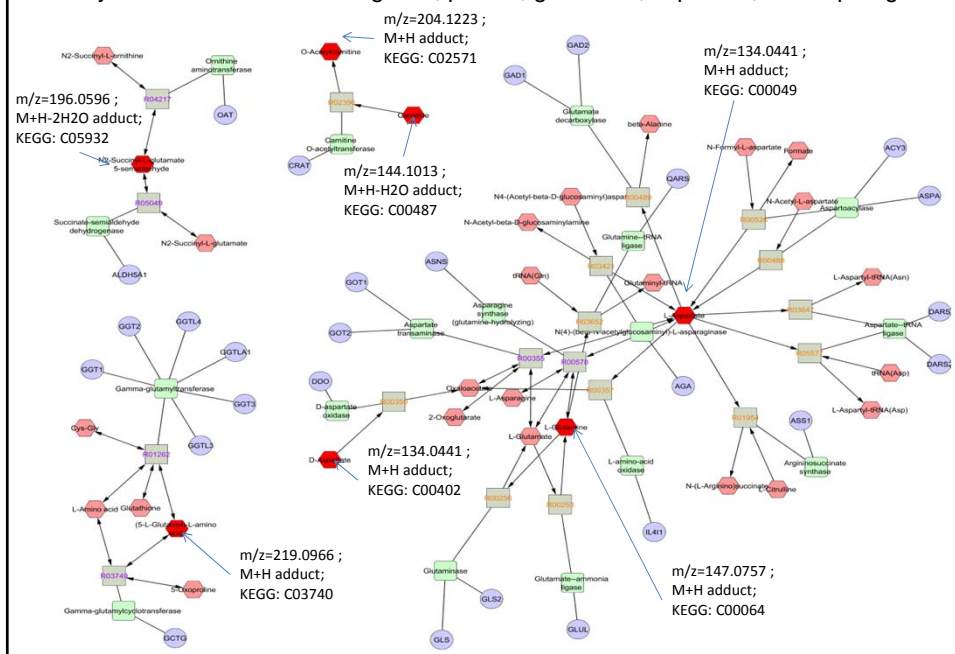


# Acetylcholine biosynthesis and metabolism



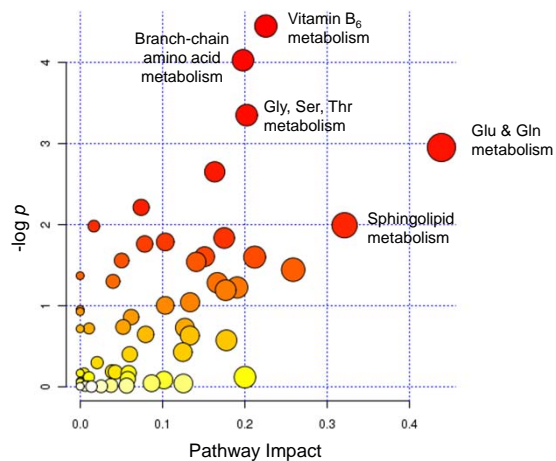
# Metscape: pathway mapping of discriminatory metabolites in disease

Urea cycle and metabolism of arginine, proline, glutamate, aspartate, and asparagine

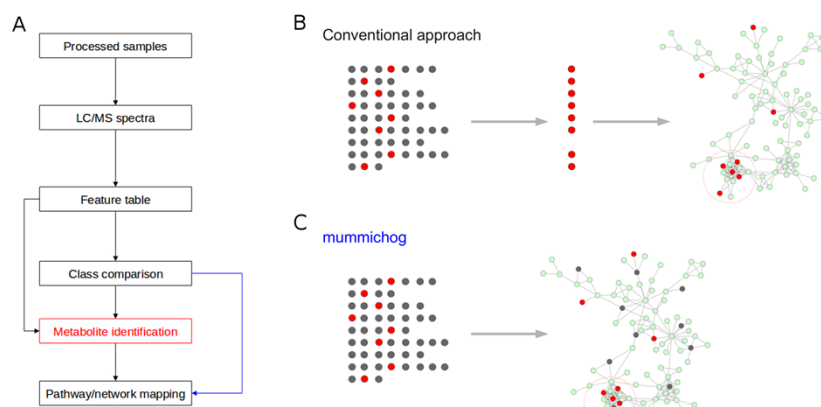


MetPA provides plot of significance and impact of metabolic pathways to separation of biological classes of samples

In this MetPA analysis, 199 matches to KEGG human compounds from 335 features significant by FDR ( $q=0.01$ ) shows calculated impact of sphingolipid, vitamin B6 and amino acid metabolism

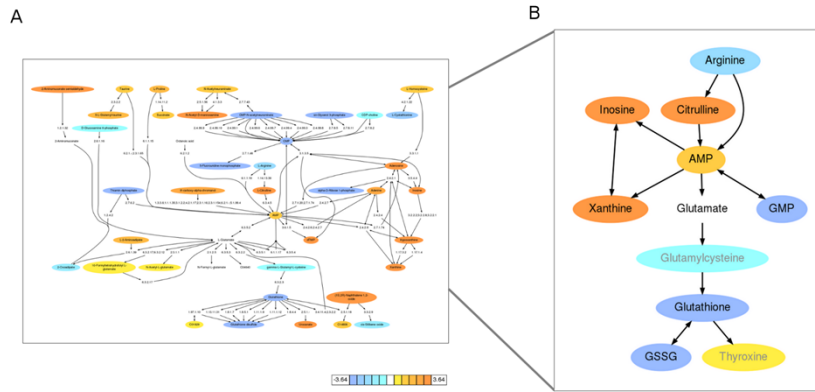


*Mummichog* combines metabolite prediction and network analysis in one step



Shuzhao Li et al, 2013 PLoS Computational Biology

Activity network predicted by *Mummichog* in innate immune activation

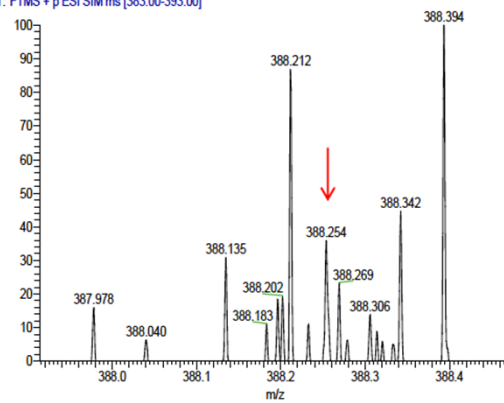


Shuzhao Li et al, PLoS Computational Biology 2013

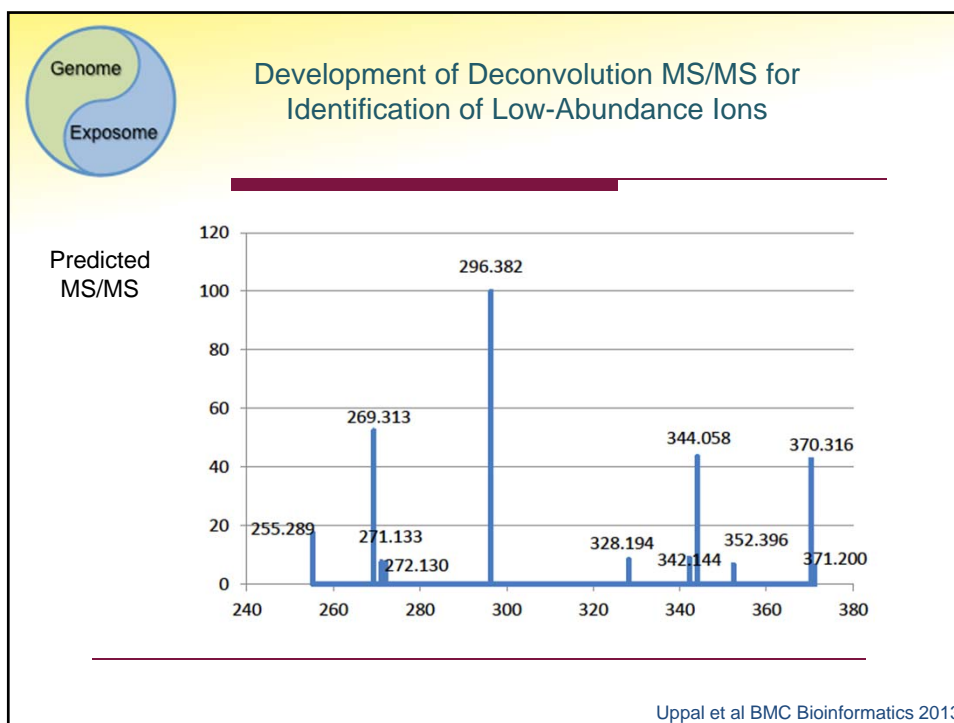


Development of Deconvolution MS/MS for Identification of Low-Abundance Ions

QStdRun1 MSMS #187 RT: 3.52 AV: 1 NL: 4.66E2  
T: FTMS + p ESI SIM ms [383.00-393.00]



Uppal et al BMC Bioinformatics 2013



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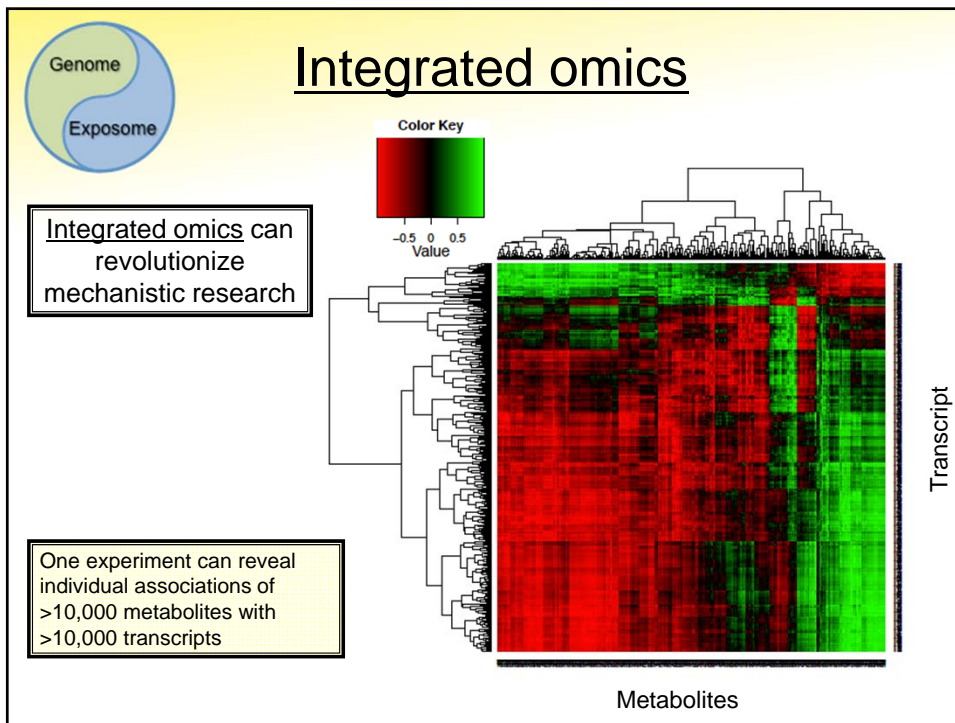
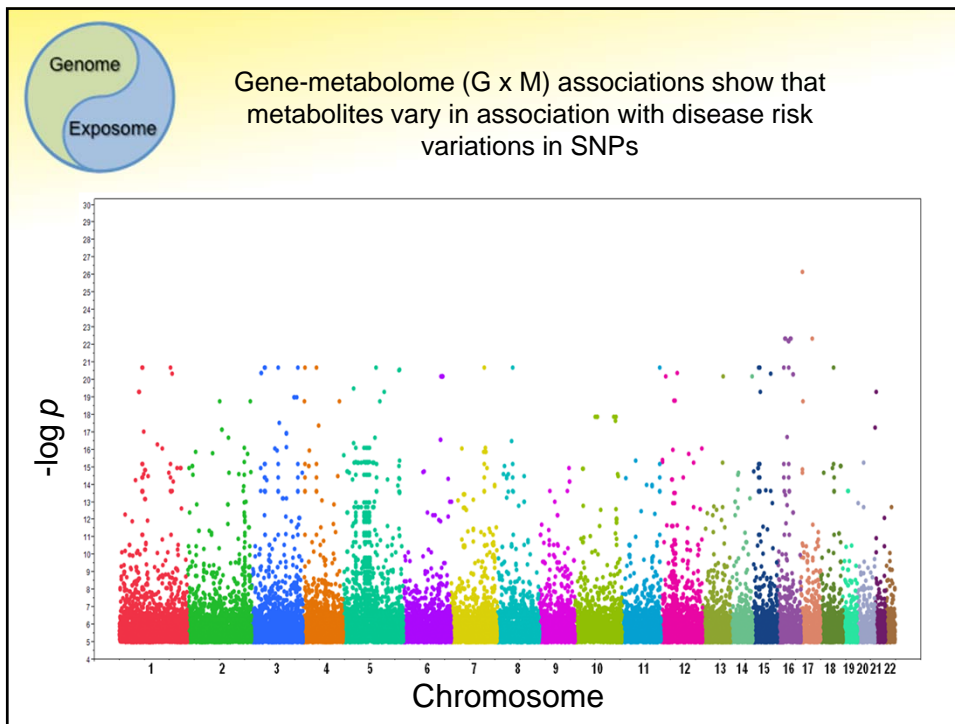
## Integrated omics for pathway analysis

**Genome x Metabolome**

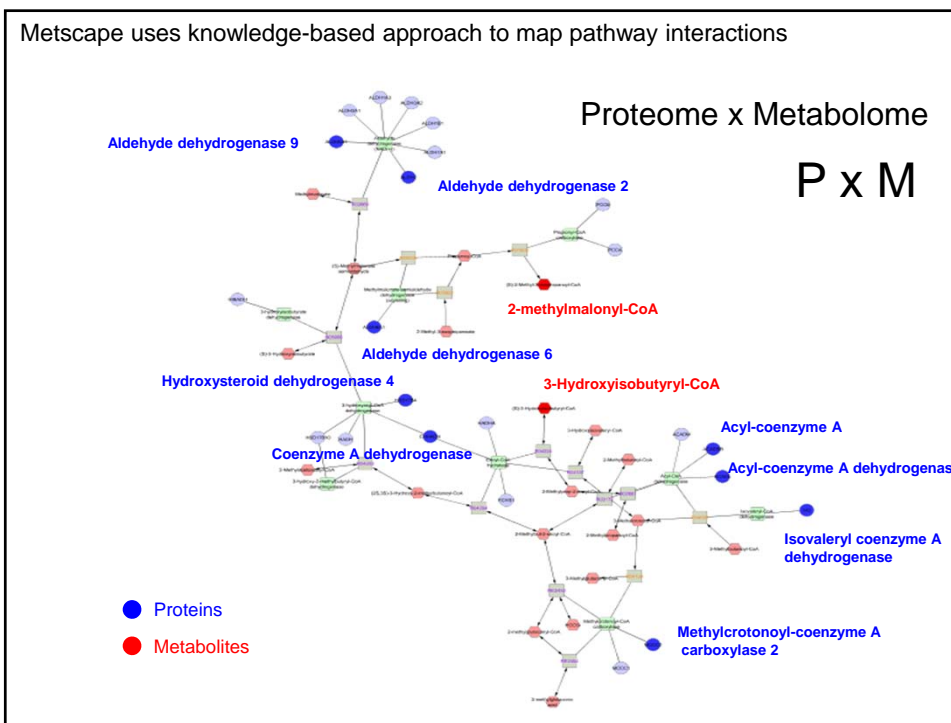
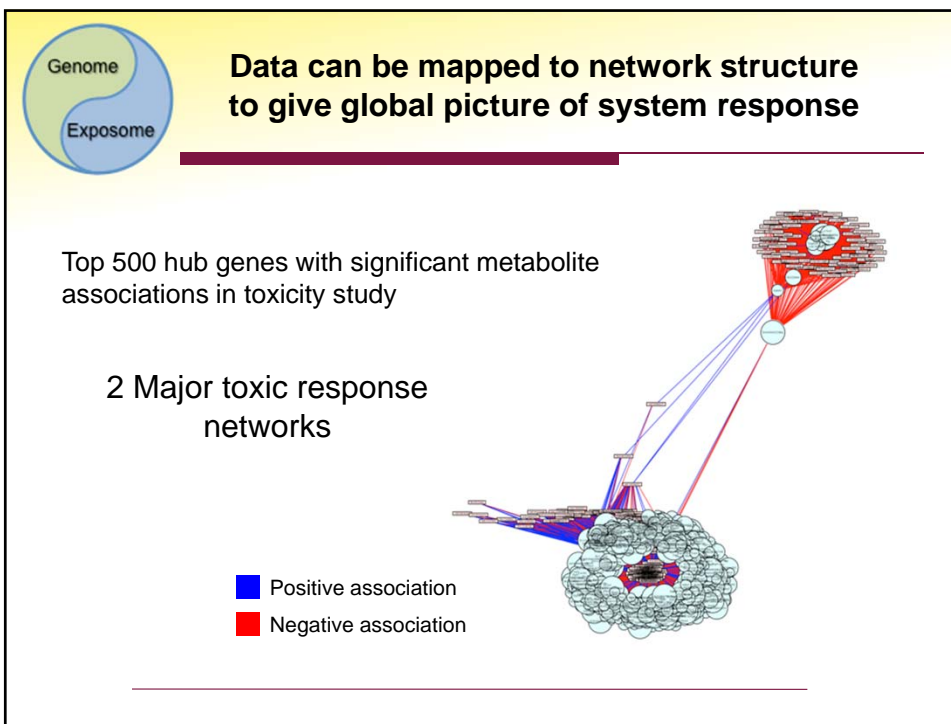
**Transcriptome x Metabolome**

**Proteome x Metabolome**

  
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### Toward a Surveillance & Forecasting System for Personalized Medicine

Have an affordable system using metabolomics to


**forecast risk of disease, timing of disease onset and intensity of impact**

and use this system to

**Improve disease prevention, classification and treatment**



The map shows the path of Hurricane Sandy, labeled "Sandy" with a red "1" in a circle. It tracks from the Atlantic Ocean towards the Caribbean Sea. Two curved lines represent the "Model Western Edge" and "Model Eastern Edge". The area between these edges is divided into "Less Certain Long-Term" (top) and "Certain Short-Term" (bottom). Other labels include "Bermuda", "The Bahamas", "Turks and Caicos Islands", "Virgin Islands", and "Anguilla". A scale bar indicates "100 pm CDI 10/26/12".



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