“What is the Metabolome and How Can it Improve Understanding of Disease Mechanisms?”

Application to the WHI Study

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I. The Metabolome and Its Measurement

II. The Metabolome in CVD

III. The Metabolome in Ovarian Cancer
**Metabolome** – all metabolites of a biological system which are end products of cellular processes.

2007 Human Metabolome Project 1\textsuperscript{st} Draft of Human Metabolome - 2500 metabolites, small-molecules - 50-1500 mw.

- **Endogenous** – produced by host and its microbiome.
- **Exogenous** – eg. food, drugs.
Methods – 2 Steps

- **Separation**
  - Gas chromatography (GC)
  - High Performance Liquid Chromatography (HPLC)
  - Ultra Pressure Liquid Chromatography (UPLC)

- **Detection**
  - Mass Spectrometry (MS) gives mass spectral fingerprint, can identify metabolite from “library” of known reference compounds.

*Used in tandem*
Metabolomics Overview

Science, Dec 2010: “Metabolism is not Boring!”

- Bio-Samples (Serum, Urine, Tissue, etc.)
- Sample preparation (Extraction, derivatization for NMR, GC-MS, etc.)
- 1D/2D NMR (1D WOESY, 2D TOCSY, HS-MAS, etc.)
- 1D/2D MS (LC-MS, GC-MS, DESI-MS, etc.)
- Data preprocessing (Baseline correction, binning, scaling, etc.)
- Statistical analysis
- Putative Biomarkers (Identity, quantity)
- Validation and Biological Understanding
- Clinical Trials
Other “OMICS”

- **Proteomics - Proteins**
  - Nuclear Magnetic Resouce
  - Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF)

- **Lipidomics**

- **Glycomics**

Along with metabolomics, used as phenotype tool, map to biochemical pathways for better understanding health and effects of genes, microbiome, diet, lifestyle and drug treatment.
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Studies of the Metabolome in Cardiovascular Disease
Wang Z, et al. (S. Hazen’s Group)


Experimental Design

1) Non-targeted metabolomics to identify metabolites associated with CVD. (Survey – hypothesis generating)

2) Targeted metabolomics - pathway identified.

3) Determine source of metabolites - diet/microbiome/liver.

4) Explore mechanisms - cell culture, mouse studies.

5) Intervention in mice.
Findings

1) Non-targeted metabolomics of plasma showed 18 metabolites, including phosphatidylcholine, betaine and trimethylamine oxide (TMAO) elevated in CVD patients.

2) Targeted metabolomics showed choline, betaine and TMAO predicted risk for CVD.

3) Source – diet (eggs, marine fish, seafood, cheese, meat).
4) **Mechanisms**

- Mice fed choline or TMAO had greater atherosclerosis.
- Suppression of gut flora in choline fed mice suppressed atherosclerosis.
- Genetic control of flavin monooxygenases (FMO) altered atherosclerosis in mice fed choline.

5) **Intervention**

- A small molecule inhibitor of TMAO reduces atherosclerosis in mouse model.
Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis

Graphical Abstract

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In Brief
Drugging the gut microbiota with a non-lethal inhibitor that blocks production of the metabolite trimethylamine reduces the formation of atherosclerotic lesions and represents the first step toward treatment of cardiometabolic diseases by targeting the microbiome.
Remaining Questions

1) Is TMAO a mediator or marker of CVD in man?

Elevated plasma TMAO maybe a marker for a gut microbiome that has a deleterious effect on health.

2) Are changes in the plasma TMAO related to diet? Or to bacterial composition of gut?
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Metabolic Profile of Ovarian Cancer
(Szyperski T, Odunsi K, Genco R, 2017)

**Purpose:** To identify metabolic profiles that may be related to ovarian cancer development; markers for ovarian cancer.

**Design:** Case-control-discovery study:
Healthy (n=40)
Benign tumor (n=40)
Ovarian Cancer (n=40)
**Method** – Analysis by Metabolon, Inc.

- Serum samples extracted with solvent
- Analyses by GC/MS
- Analyses by LC/MS-MS

**Controls**

- Pool of all above in all runs
- Internal standards SD (4%)
- Endogenous markers SD (16%)
Results of Untargeted Metabolomics
Statistically Altered Metabolites

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Metabolites ↑ or ↓</th>
</tr>
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<tbody>
<tr>
<td>Benign vs Healthy</td>
<td>28/37</td>
</tr>
<tr>
<td>Cancer vs Healthy</td>
<td>21/33</td>
</tr>
<tr>
<td>Cancer vs Benign</td>
<td>20/44</td>
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Metabolites from Gut Microbiome Altered

9 others detected.
Main Findings

- Increase in metabolites associated with hyperglycemia and hyperlipidemia in cancer patients.
- Increase in serum clotting factor peptides in cancer patients.

NOTE:

- CA-125 values inferior to metabolite profiles as diagnostic for ovarian cancer.
- Alterations in microbiome – associated metabolites.

This Pilot justifies a major targeted metabolomic study to validate findings on another population.

Is this the type of study that can be done on WHI Observational Cohort? Do baseline target metabolites predict incident ovarian cancer?
Opportunities

1) Study of metabolites in WHI has great potential to identify markers, as well as elucidate etiopathological mechanisms for disease.

2) Metabolomics as well as analyses of the microbiome, proteome, lipidome, and glycome can be utilized to reveal important metabolic pathways.

3) The WHI longitudinal design and large size are strengths to identify metabolites as disease predictors, and participants in etiologic pathways.

4) WHI intervention studies also can be used to identify important metabolic pathways (eg. diet study).
Major Collaborators

Jean Wactawski-Wende
Michael LaMonte
Thomas Szyperski
Adekunle Odunsi
Stanley Hazen
Thank You!