Discovery-Based Nutritional Systems Biology
Developing n-of-1 Biomedical Research Designs

Veyrier du Lac, France
14 September 2012
Mission Statement

To create and deliver world class excellence in biomedical research to better understand human health and ageing as influenced by genetics, metabolism and environment with the goal of translating this knowledge into personalized science-based nutrition.
...food be your medicine

...aptitude of the body

...adapt to the environment

...inborn errors of metabolism

...biochemical individuality

19th & 20th Century Science

21st Century Science: n-of-1

Strategy

Homeostatic Challenges (another time)

Long Term Interventions

Vitamin Project

Next Steps
### Facts & Challenges 20\textsuperscript{th} Century Science

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pheno</th>
<th>Pheno</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

A,B,C = variants of one gene or A,B,C = variants of many genes

\(\Sigma\) Phenotype / 16 = Average Phenotype

\(\Sigma\) Phenotype / 14 = Average Phenotype

Nestlé Institute of Health Sciences
...food be your medicine

19th & 20th Century Science

21st Century Science: n-of-1

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Conceptual Basis

A different effect of a *genotype* on disease in persons with different *environmental* exposures

A different effect of an *environmental* exposure on disease risk in persons with different *genotypes*


| Statistical Parllance | The *main effect(s)* may be *genotype x environment interaction(s)* for chronic diseases and modifying effects |
1000Genomes: 300 – 400 variants affecting 250 – 300 genes resulting in loss of function (LOF) \textit{per person}


Lu et al \textit{EJHG} 17, 967 (2009)
Facts & Challenges  19th and 20th Century Logic

Insanity
Doing the same things over and over and expecting different results
Albert Einstein

Distribution of risk

Proportion of population at specific risk

Population

Cases

Limited variations may reduce effect size

Human genetic, nutritional, physiological variations
Follow patients/subjects over time – evaluate

- Homeostatic assessments (clinical + omic)
- Diet and lifestyle assessments
- Genomic (once) & Epigenomic
- Changes in biomarkers due to medical or lifestyle interventions (acute or long term)

Associate changes/outcome in quantitative assessments in the context of individual genomes
Experimental Design Concept n-of-1 to group level

Classification Algorithms

1, 2, 3…n = Genes.  A, B, C…n = Environment
…food be your medicine

…aptitude of the body

…adapt to the environment

…inborn errors of metabolism

…biochemical individuality

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Strategies Human Study 2 – CBPR Translational Research

Community Based Participatory Research
Research, education, translation in real time

With USDA – ARS, now COSBI, NIH

Consistent with Nestle Program: Creating Shared Value

McCabe-Sellers et al. Omics 12, 263 (2008)
Delta Vitamin Obesity Project

Metabolites in blood @ pre, end, post-intervention
Genomics, DNA methylation

Sequence micronutrient metabolism genes

Diet Intakes – 24 hr  Activity – Body Bugg  Skin tone – Dermometer

Correlate Δ metabolite(s) to an individual’s genotype

In kids and parents

Projected Levels

Start  End  After

Quantitative assessment of efficacy per individual
N-of-1 Data Aggregation Population & Group

SAM/SAH Ratios: Aggregation to Discovered Group

Cluster 1  Cluster 2

<table>
<thead>
<tr>
<th>Metab</th>
<th>1*</th>
<th>2*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 10x</td>
<td>n = 52x</td>
<td>(between)</td>
</tr>
<tr>
<td>SAM</td>
<td>1.44</td>
<td>0.77</td>
<td>&gt; 0.000</td>
</tr>
<tr>
<td></td>
<td>nm/ml</td>
<td>nm/ml</td>
<td></td>
</tr>
<tr>
<td>SAH</td>
<td>0.88</td>
<td>0.96</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>nm/ml</td>
<td>nm/ml</td>
<td></td>
</tr>
<tr>
<td>M/H</td>
<td>1.81**</td>
<td>0.81**</td>
<td>&gt; 0.000</td>
</tr>
</tbody>
</table>

* Center cluster 1 to cluster 2 distance = 0.991
** Reference = 1.40

http://www.youtube.com/watch?v=2kYRvABhHAY
n-of-1 Analyses for Population, Group, & Individual

Use common statistical methods to place individual into high, medium, low groups.
Hierarchical Cluster Analyses of Plasma Metabolites & RBC SAM & SAH

Bar = mean of tertile level of metabolite

n-of-1 & Aggregation
Row = individual
Cluster = group
Column = population
Principal Components  Micronutrient Profile

- Component 1 (41%)
- Component 2 (15%)

- Vitamin A
- SAM:SAH
- Homocysteine
- Vitamin D
- Riboflavin
- Thiamine
- Pyridoxal
- Vitamin E
- Vitamin B6

- Variances
- Component
## Individual Level Nutrient Intake by Year - 2 Participants

<table>
<thead>
<tr>
<th>Intake Measure 3X 24hr Diet Survey</th>
<th>High SAM/SAH¹</th>
<th>Child 1</th>
<th>Child 2</th>
<th>Low SAM/SAH²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td>Kcal/kg</td>
<td>67.1</td>
<td>111.6 (49)</td>
<td>52.4 (46)</td>
<td>79.6 (65)</td>
</tr>
<tr>
<td>HEI Total grain</td>
<td>4.2</td>
<td>5.0</td>
<td>4.1</td>
<td>4.6</td>
</tr>
<tr>
<td>HEI Milk</td>
<td>7.3</td>
<td>3.5</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>HEI Saturated Fat</td>
<td>4.1</td>
<td>6.0</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>HEI SOFAA</td>
<td>4.9</td>
<td>3.0</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Protein</td>
<td>61.6</td>
<td>100.3 (34)</td>
<td>57.3 (34)</td>
<td>49.3 (19)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>250.1</td>
<td>394.0 (130)</td>
<td>264.9 (130)</td>
<td>221 (130)</td>
</tr>
<tr>
<td>Sugar</td>
<td>123.3</td>
<td>164.7</td>
<td>169.4</td>
<td>95.8</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>27.3</td>
<td>38.7</td>
<td>28.8</td>
<td>26.6</td>
</tr>
<tr>
<td>Thiamine</td>
<td>1.5</td>
<td>2.4 (0.9)</td>
<td>1.5 (0.9)</td>
<td>1.2 (0.6)</td>
</tr>
<tr>
<td>Folate DFE</td>
<td>420.9</td>
<td>522.1 (300)</td>
<td>386.7 (300)</td>
<td>341.5 (200)</td>
</tr>
<tr>
<td>Iron</td>
<td>13</td>
<td>18.9 (8)</td>
<td>12.4 (8)</td>
<td>10.1 (10)</td>
</tr>
<tr>
<td>Sodium</td>
<td>3420.9</td>
<td>5588.5 (1500)</td>
<td>3363.3 (1500)</td>
<td>3043.7 (1200)</td>
</tr>
<tr>
<td>Selenium</td>
<td>86.5</td>
<td>153.0 (40)</td>
<td>71.1 (40)</td>
<td>92.5 (30)</td>
</tr>
</tbody>
</table>

¹,² Designate average of values in cluster 1 and 2 respectively.

Values in ( ) are Recommended Dietary Allowances (RDA) or Adequate Intake (AI) references but not individual requirement.
### Individual Level Metabolites by Year - 2 Participants

<table>
<thead>
<tr>
<th>Plasma Mean</th>
<th>High SAM/SAH</th>
<th>Child 1</th>
<th>Child 2</th>
<th>Low SAM/SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td>Vit A</td>
<td>84.2</td>
<td>84.6</td>
<td>36</td>
<td>110.7</td>
</tr>
<tr>
<td>Vit E</td>
<td>1.8</td>
<td>1.2</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Vit D</td>
<td>24.6</td>
<td>29.3</td>
<td>17.3</td>
<td>25.3</td>
</tr>
<tr>
<td>Pyridoxal</td>
<td>5.2</td>
<td>0.1</td>
<td>15.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>9.7</td>
<td>5.8</td>
<td>1.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Vit B2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>11.8</td>
<td>12.6</td>
<td>7.2</td>
<td>11.9</td>
</tr>
<tr>
<td>SAM</td>
<td>1.5</td>
<td>1.3</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>SAH</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>SAM/SAH</td>
<td>1.8</td>
<td>1.5</td>
<td>0.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

1.2 Designates average of values in cluster 1 and 2 respectively

3 Retinol = 30 – 80 μg/dl

4 Vit E = 0.5 – 1.8 mg/dl

5 Vit D = 14 – 60 ng/ml

6 B6 Pyridoxine = 5 – 30 ng/ml (I do not have pyridoxal reference)

7 Riboflavin (B2) = 4 – 24 μg/dl

8 Folate = 3.0 – 20.0 ng/ml in serum

9 Homocysteine values between 15-30 μmol/l mean lack of vitamins

Values between 31-100 μmol/l indication of heterocyste homocysteinemia;
Values > 100μmol/l indication of homocyste homocysteinemia.
Middle Out Micronutrient Genomics System

MICRONUTRIENTS
- Retinoic acid
- Folic acid
- Homocysteine
- Pyridoxal
- Pyridoxine
- Riboflavin
- s-adenosyl methionine
- s-adenosyl homocysteine
- Thiamine
- Vitamin D
- Tocopherol

GeneGO query

 MICRONUTRIENT GENES (n=275)

Topological partitioning Enrichment analysis

GLOBAL INTERACTION NETWORK
13705 genes
100075 interactions

MICRONUTR ENRICHED MODULES
1580 genes
17901 interactions
16208 SNPs

Nestlé Institute of Health Sciences
Gastric acid secretion Module 18
Individual Level Ancestry Mapping of Significant Genes
Limitations and Advantages Delta Study & Middle Out

Limitations – small sample size

Genetic spectrum is narrow (admixture an advantage)
Cultural and food diversity is constrained
Physiology of individual not well defined
Middle out not yet optimized

Advantages for new strategy – systems biology

Population level data by aggregating individual data
Metabolic group level data by discovery methods
Individual level data
Outcomes raised new nutrition/health questions
Middle out may identify genetic patterns
Summary 21st Century Science

Analyze genome – no randomization

Response: acute / long-term intervention

Measure then classify

Middle – out strategies for complex systems (interim)

Compare (genetics and lifestyle)

Systems biology concepts & approaches

CBPR translates research: near & long term health benefits
New Intervention Studies  Partnering to Effect Change

Local, NGOs & Partners

Community Based
1. Participatory
2. Ethics
3. Health assessments
4. Education
5. Built environment
6. Shared value

NuGO ** & Collaborators

Translational Research
1. MMMV intervention
2. Harmonized
3. Omic analyses
4. Longitudinal
5. Lifestyle measures
6. Participatory

COHRED & Partners

Economic Analyses
1. Of community
2. Prevention research
3. Capacity building
4. Sustainability
5. Partnerships
6. Policies

** Nutrigenomics Organization
Carolyn Wise
Anna Williams
Rick Beger
Lisa Pence

Jacqueline Monteiro (USP-RP)

Beverly McCabe - Sellers
Margaret Bogle
Dalia Lovera
Catherine Champagne (PRC)

National Center for Toxicological Research
Division of Personalized Nutrition & Medicine
Food and Drug Administration

Jerome Turner
Beatrice Shelby
Community members
Middle Out  Micronutrient Genomics System
Middle Out Metabolic/PPI network

- 13705 nodes
- 116210 interactions
- 125884 SNPs
- 58 modules
Middle Out  Micronutrient Genomics System

GLOBAL INTERACTION NETWORK
13705 genes
100075 interactions

MICRONUTRIENT GENE ENRICHED MODULES
1580 genes
17901 interactions
16208 SNPs

Topological partitioning
Enrichment analysis
**Middle Out** Enriched module – Genotype pattern

Freq > 0.5%
Homozygote = 0
Heterozygote = 1
Homozygote = 2

Module 18: 465 genes
Expect 8, found 42 (Micro Profile)
Corrected modular p = 2.63E-19
QUANTITATIVE TRAIT LOCI PHENOTYPES ASSOCIATED WITH GENEWISE SAM:SAH or/and MICRONUTRIENT PROFILE

102 Allergic/atopic asthma
147 blood pressure
252 Body weight
35 Fibrinogen
28 Glucose level
14 Rheumatoid Arthritis
33 Serum adiponectin
5 Serum Gherlin
39 Serum Leptin
18 Serum P-Selectin

GLOBAL INTERACTION NETWORK
13705 genes
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Middle Out  Micronutrient Genomics System

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- Retinoic acid
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- Pyridoxal
- Pyridoxine
- Riboflavin
- s-adenosyl methionine
- s-adenosyl homocysteine
- Thiamine
- Vitamin D
- Tocopherol

GeneGO query
Middle Out Level 1 Pathway & Network (274 micro genes)

Freq > 0.5%

Homozygote = 0

Heterozygote = 1

Homozygote = 2

274 Genes

SAM:SAH

44 Genes

Corrected for age, gender, sibship and mean HEI
Connecting QTL and PPI

Human body weight QTLs

MODULE 18

MODULE 47

- chromosome
- genes in gastrointestinal secretion pathways
- genes significantly correlated with PCI AND containing body weight QTL
- interactions between genes in module

chromosome
genes significantly correlated with PCI AND containing body weight QTL
genes in gastrointestinal secretion pathways
interactions between genes in module
19th & 20th Century Science

21st Century Science: n-of-1

Strategy
Homeostatic Challenges (another time)
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Next Steps
Thank You and Input

Choose ancestors wisely

Move more

Eat less