Microbiome and Metabolome Studies: Overall WHI, Buffalo Clinical Center, and Future Directions

Mike LaMonte
University at Buffalo

WHI Investigators Meeting
May 4-5, 2017
Columbus, Ohio
Betsy:
The **Microbiome**: definition, measurement and role in epidemiologic research.

Bob:
What is the metabolome & how can it improve understanding disease mechanisms?

Some Studies Completed or Ongoing in WHI

- **WHI BAA-24**: Metabolomics of CHD in WHI (Kathryn Rexrode)
- **Cancer Res 2014**: Plasma Choline Metabolites and Colorectal Cancer Risk (Sajin Bae)
- **WHI AS531**: Feasibility study of the fecal (gut) microbiome (Mike LaMonte)
- **SUNY Buffalo Funds**: Feasibility study to measure methylglyoxal (Mike LaMonte)
- **WNY MI-Perio Study**: Serum TMAO, Periodontitis and CVD risk (Bob Genco)
- **NIDCR R01**: Buffalo OsteoPerio Microbiome Study (Jean Wactawski-Wende)
- **Other Metabolomics work in WHI** (Simin Liu; Dan Rafftery, Others ...)
Study Aim was to complete targeted and untargeted metabolomics on serum samples in order to determine relationships between individual metabolites and incident CHD in older women.

Presented at 2017 AHA Epidemiology Council: *Metabolomics Profiles and CVD Mortality in Women*

943 OS, 1355 HT women (median age 68), median follow-up 16.7 years (1,102 total deaths; 601 CVD)

Significant metabolites were discovered in the WHI-OS, then validated in the WHI-HT. A composite score was developed using 11 metabolites significantly associated with total mortality after mutual adjustment for one another.

Comparing highest to lowest quartile of the score, the multivariable adjusted HR (95% CI) was:

- 2.59 (1.94-3.47) for total mortality; 2.67 (2.07-3.44) for CVD mortality

Individual metabolites associated with both endpoints included *lipid metabolites, amino acids, eicosanoids and uracil metabolites*

*Kathy says “stay tuned for more results!”*
Study Aim to examine choline metabolites (choline, betaine, DMG, TMAO) and CRC risk.

835 incident CRC cases, 835 controls matched on age, race-ethnicity, timing of baseline blood draw, enrollment date, hysterectomy status.

Multivariable OR (95% CI) comparing quartile extremes was:

- 0.56 (0.39, 0.82) for choline:betaine ratio & overall CRC; 3.38 (1.25, 9.16) for TMAO & rectal cancer

Results suggest a potential role for gut microbiome in CRC pathogenesis.
Feasibility Study to Measure Methylglyoxal (MGO), TMAO

Mike LaMonte, PhD; Bob Genco, DDS, PhD
University at Buffalo

- MGO highly reactive glycating agent (AGEs)
- Animal & human studies show MGO associated with LDLox, & insulin resistance
- Potential role in atherosclerotic CVD, diabetes
- MGO is secreted by Tannerella Forsythia, a pathogenic periodontal bacteria
Feasibility Study to Measure Methylglyoxal (MGO), TMAO

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• Western NY MI-Perio case-control study (1997-2001)
• 638 acute incident MI cases, 965 matched controls
• MGO Feasibility Study
  • Method development phase (Rick Browne, PhD)
  • Validate analytic method to measure serum MGO adducts
  • Proof of Concept phase
  • MGO measured in Tannerella f. culture
  • Compare serum MGO between those with/without diabetes, periodontitis, MI
• TMAO Feasibility Study
  • 168 index MI, 168 recurrent MI, 168 controls
  • Compare serum TMAO between groups
  • 16S rRNA sequencing of saliva, subgingival plaque for bacteria capable of producing TMAO

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Atheromatous plaque from carotid endarterectomy
Targeted culture-dependent assay
P. gingivalis, Tanneralla f. abundant
Microbiome of Atheromatous Plaque Pilot Study
Mike LaMonte, PhD; Bob Genco, DDS, PhD
University at Buffalo

Identification of Periodontal Pathogens in Atheromatous Plaques
V.I. Haraszthy,1 J.J. Zambon,2 M. Trevisan,2 M. Zeitlin,2 and R.J. Genco*3

Recent studies suggest that chronic infections including those associated with periodontitis increase the risk for coronary vascular disease (CVD) and stroke. We hypothesize that oral microorganisms including periodontal bacterial pathogens enter the blood stream during transient bacteremia where they may play a role in the development and progression of atherosclerosis leading to CVD.

Methods: To test this hypothesis, 50 human specimens obtained during carotid endarterectomy were examined for the presence of Chlamydia pneumonia, human cytomegalovirus, and bacterial 16S ribosomal RNA using specific oligonucleotide primers in polymerase chain reaction (PCR) assays. Approximately 100 ng of chromosomal DNA was extracted from each specimen and then amplified using standard conditions (25 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 30

Recent data suggest that chronic infection may play an important role in the development of atherosclerosis. This concept arises from the fact that traditional risk factors for atherosclerosis are common to both chronic infections and connective tissue disease.

• Atheromatous plaque from carotid endarterectomy
• Targeted culture-dependent assay
• P. gingivalis, Tannerall f. abundant

• Study Aim to compare the atheroma, oral and gut microbiomes
• Aorta sample during clinically indicated CABG (Hashmet Ashraf, MD)
• Subginvial plaque, feces obtained during week prior to CABG
• 16S rRNA sequencing of the atheroma, plaque, feces samples to characterized microbiome
• Functional measurement (Shotgun sequencing) to determine strain comparability between samples, and to identify metabolic pathways associated with microflora
Buffalo OsteoPerio Microbiome Study
Jean Wactawski-Wende, PhD
University at Buffalo

• Buffalo OsteoPerio Study, ancillary to WHI OS at Buffalo clinic site.
• Baseline (1997-2001) enrolled 1,362 women (out of 2249 OS enrollees)
• Samples collected: fasting blood; saliva; subgingival plaque
• Clinical oral examination; oral radiographs (oral bone assessment)
• DXA, anthropometrics, medication inventory, FFQ, health questionnaires
Comparison of 16S rRNA sequencing of subgingival plaque samples between women with none/mild and severe periodontal disease at OsteoPerio Study baseline

(LaMonte et al. In review)

- 16S rRNA sequencing completed using 454 Pyrosequencing method
- Taxonomic analysis of OTU data (e.g., below panel) to characterize microbiome
- PiCrust Functional analysis (e.g., right panel) to identify bacteria possessing genes with specific metabolic functions
Comparison of Bacterial Relative Abundance in Women With and Without Periodontitis
**Buffalo OsteoPerio Microbiome Study**
Jean Wactawski-Wende, PhD
University at Buffalo

- Baseline (1997-2001) enrolled 1,362 women (out of 2249 OS enrollees)
- Fasting blood; saliva; subgingival plaque; oral examination & radiographs; DXA, anthropometrics, health Qx

- 5-Year reexamination (2002-2006) enrolled 1,025 women (out of 1341 eligible from baseline)
- Same sample collection, examinations

- 15-Year reexamination (2015-present) enrolling 600 women (with baseline & 5-Year exams)
- Same sample collection, examinations .... Also collecting feces, vaginal, nares, oropharynx, skin

- Currently completing 16S rRNA sequencing (MiSeq method) taxonomic analysis, PiCrust functional analysis
- Aim 1: Characterize the cross-sectional subgingival microbiome at Yr1, Yr5, Yr15, and its relation with perio
- Aim 2: Characterize the longitudinal changes in microbiome, and its relationship with perio progression
Additional Studies Planned:

• OsteoPerio Microbiome Study: additional data resources
  - Microbiome(s) relationship with nutrient intake and diet patterns; DXA body composition; physical performance measures; other disease endpoints via continued WHI follow-up

Current Ongoing Studies:

• Oral Microbiome and Hypertension Incidence (Josh Gordon MD/PhD student; NHLBI funded)
  - Interested in Nitrate/Nitrite oral bacteria (subgingival plaque, saliva)

• Oral Microbiome and Incident Cancer Case-Control Study (Xiaodan Mai, MBBS, PhD)
  - Tumor block collection; compare microbiome in oral and tumor tissue (tumor and adjacent normal)
Possibilities for new studies in WHI

1. **Biologic Samples:**
   - Existing serum samples (metabolome)
   - Existing tumor blocks (microbiome, metabolome)
   - Collect new samples (serum, feces, saliva, other?) … microbiome, metabolome

2. **Study Cohort:**
   - Nested designs for specified outcomes (i.e., incident diabetes, CVD, cancer)
   - CT: baseline → Yr 1 changes
     - **DM:** change in metabolome with dietary modification during Yr 1?
     - **HT:** change in thrombosis-related metabolome?
   - **WHISH, COSMOS Trials** (post-randomization sample collection & comparison)
     - Evidence exists that both physical activity and cocoa effect gut microbiome
   - **Buffalo OsteoPerio Study** could be used for feasibility data
Feasibility of Remote and Large Sample Collection in an Epidemiologic Study

Health Professionals Follow-up Study

Feasibility of Remote Self-Collection and Comparison of Processing Methods

Franzosa EA et al. *PNAS* 2014 doi/10.1073/pnas.1319284111
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WHI women have demonstrated willingness to provide samples:

- Yunsheng Ma’s pilot study of mail collection of blood cards
- Buffalo OsteoPerio Study collecting feces (at home, mix with Mo Bio), saliva, blood
- Buffalo OsteoPerio Study cancer feasibility study (tumor blocks)/ WHI Lilac
- LLS home-visit, blood collection; OPACH mail return accelerometers (>90%)
- COSMOS remote blood collection

23andMe ... as of 2015, more than two million samples tested worldwide
Exercise is a Novel Promoter of Intestinal Health and Microbial Diversity

Opportunities within WHISH and COSMOS Trials?

Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study\textsuperscript{1,3}


Xenophon Tzounis, Ana Rodriguez-Mateos, Jelena Vulevic, Glenn R Gibson, Catherine Kwik-Uribe, and Jeremy FE Spencer

Mean (SD) log10 bacterial numbers per gram feces
Possibilities for new studies in WHI

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3. **Your Ideas?**
Bioinformatics Pipeline

sampling → DNA extraction → sequencing → sequences

taxonomy independent analysis

composition analysis: to estimate biodiversity
Taxonomy Independent Analysis

OTU: Operational Taxonomic Unit
3%: species, 5%: genus, 20%: phylum

profile data matrix