EPIDEMIOLOGICAL INVESTIGATIONS IN UNDERSTANDING HEALTH IMPACTS OF HUMAN-BUILT ENVIRONMENT INTERACTIONS

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Microbiome of Built Environment

Multiple Microbial Exposure Biomarkers
Gram – bacteria: Endotoxin, 3-OHFA
Gram + bacteria: Muramic acid
Fungi: Ergosterol, (1→3) Beta-D Glucan

Culture Data

Microbiome Sequencing
16S
ITS
Shotgun metagenomics

Human Health Outcomes

Environmental Health
Infant/Childhood Health
Wheeze, Asthma
Allergen Sensitization
Immune Outcomes

Adult Health
Asthma Severity

Occupational Health
Airway Obstruction
Cancer
Microbiome of Built Environment

Human Microbiome

Building Characteristics / Human Behaviors

Human Health Outcomes
PET OWNERSHIP, HOME DAMPNESS, CLEANING AND RACE ARE ASSOCIATED WITH HOME MICROBIAL BIOMARKERS

Dog Ownership (Can f 1 ≥ 20µg/g)

Water Damage/Visible Mold

HAA Cohort, Bed dust

Sordillo et al, EHP (2011)
PET OWNERSHIP, HOME DAMPNESS, CLEANING AND RACE ARE ASSOCIATED WITH HOME MICROBIAL BIOMARKERS

Cleaning Frequency (≥ 1 x per week)

<table>
<thead>
<tr>
<th>Percent_change</th>
<th>Fungi (Ergosterol)</th>
<th>Gram - (3-OHFA)</th>
<th>Gram - (rFC)</th>
<th>Gram + (Muramic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAA Cohort, Bed dust</td>
<td></td>
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</table>

Black Race/ethnicity

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<th>Percent_change</th>
<th>HAA Cohort, Bed dust</th>
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<td>Gram - (rFC)</td>
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XENOBIOTIC DEGRADATION OF HOUSEHOLD CHEMICALS MAY BE OVER-REPRESENTED IN INDOOR BACTERIA

- Pilot Study of Boston-Area Home Dust Samples (N=12) showed over-representation of caprolactam, limonene, pinene, and geraniol degradation (PICRUST imputed functional metagenome from 16S)

**Imputed Functional Pathways also include Degradation of:**

- Pesticides (DDT, Atrazine)
- Bisphenol A
- Dioxin
- Polyaromatic hydrocarbons
- Fluorobenzoate

Hanson et al, Envir Sci Processes Impacts, (2016); JAX Collaboration, Sodergren/Weinstock lab
DIFFERENCES IN BACTERIAL AND FUNGAL COMMUNITIES OBSERVED BY HOME CHARACTERISTIC

Dannemiller et al (2015) study

- House dust 198 asthmatic children’s homes
- Community composition differences for AC use and occupancy (people and pets)
- Pets
  - Increased Bacteroides, Firmicutes, Gammaproteobacteria, and Fusobacteria
  - *Porphyromonadaceae, Pasteurellaceae*
- AC
  - Fungal genus *Candida*, and classes Wallemiomyces andSaccharomycetes higher
Microbiome of Built Environment

Human Microbiome

Human Health Outcomes

Building Characteristics / Human Behaviors
Microbiome of Built Environment

- **Multiple Microbial Exposure Biomarkers**
  - Gram – bacteria: Endotoxin, 3-OHFA
  - Gram + bacteria: Muramic acid
  - Fungi: Ergosterol, (1→3) Beta-D Glucan

- **Culture Data**
  - **Microbiome Sequencing**
    - 16S
    - ITS
    - Shotgun metagenomics

- **Human Health Outcomes**
  - **Environmental Health**
    - Infant/Childhood Health
      - Wheeze, Asthma
      - Allergen Sensitization
      - Immune Outcomes
  - **Adult Health**
    - Asthma Severity
  - **Occupational Health**
    - Airway Obstruction
    - Cancer
TIMING, CONSTANCY OF MICROBIAL EXPOSURE IS KEY

**EARLY LIFE EXPOSURE:**
Home Endotoxin associated w/

↑ Wheeze in infancy:
- RR= 1.33 (0.99 to 1.8) for ≥ median endotoxin in house dust¹

↓ Allergen Sensitization at school age:
- RR= 0.5 (0.2 to 0.9) for > lowest quartile endotoxin in house dust²

**LONGITUDINAL EXPOSURE:**
Home Endotoxin (consistently elevated)
↓ wheeze, ↓ asthma in mid-childhood³

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*HAA Boston-Area Birth Cohort Findings*

³Sordillo JE et al, CEA (2010)
MEASUREMENT ERROR CORRECTION TECHNIQUES MAY ENHANCE OUR ABILITY TO DETECT HEALTH EFFECTS OF MICROBES

• Airway irritant effects of endotoxin are likely the result of inhalation exposure
• Measurement error correction (Horick et al, EHP 2006), used information from a validation study (paired dust and air samples) to estimate relevant (airborne) exposure
  • 25% of homes had both dust and air (1.5 day) samples

Endotoxin and wheeze in early life (corrected vs. uncorrected associations):

<table>
<thead>
<tr>
<th>Model</th>
<th>Uncorrected</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\hat{\beta}$ ($p$-value)</td>
<td>$RR^a (95% CI)$</td>
</tr>
<tr>
<td>Univariate</td>
<td>0.84 ($&lt;0.01$)</td>
<td>1.33 (1.11–1.60)</td>
</tr>
<tr>
<td>Multivariate$^c$</td>
<td>0.89 ($&lt;0.01$)</td>
<td>1.35 (1.11–1.65)</td>
</tr>
<tr>
<td>Multivariate$^d$</td>
<td>1.09 ($&lt;0.01$)</td>
<td>1.45 (1.20–1.76)</td>
</tr>
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</table>

$^a$Estimated RR reflects an increase of one interquartile range [0.34 log$_{10}$ (EU/mg)] in dust endotoxin exposure. $^b$Estimated RR reflects an increase of one interquartile range [0.39 log$_{10}$ (EU/m$^3$)] in airborne endotoxin exposure. $^c$Adjusted for race, presence of dog in home, former (not current) dog in home, use of dehumidifier, total mass of dust sample collected (in log scale), presence of concrete floor, missingness indicator for presence of concrete floor, and presence of water damage in the measurement error model. $^d$Further adjusted for lower respiratory illness, in addition to covariates of the previous multivariate model.
CULTURABLE TAXA ARE ASSOCIATED WITH WHEEZE, BUT REPRESENT ONLY A FRACTION OF TOTAL ENVIRONMENTAL FUNGI

<table>
<thead>
<tr>
<th>Culturable Fungal Genera (CFUs/gm bedroom flr dust)</th>
<th>OR (95% CI) for Any Wheeze in the 1st year of life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>1.84* (1.12–3.02)</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>1.40* (1.14–1.73)</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>1.04 (0.86–1.26)</td>
</tr>
<tr>
<td>Penicillium</td>
<td>1.21* (1.02–1.44)</td>
</tr>
<tr>
<td>Total Yeasts</td>
<td>0.85* (0.72–0.99)</td>
</tr>
</tbody>
</table>

* p ≤ 0.05

N=316 subjects, models adjusted for for winter birth (yes/no), low birthweight quartile (yes/no), maternal smoking during pregnancy (yes/no), and maternal history of physician-diagnosed asthma (yes/no).

ITS Sequencing Data (Pilot Study)
- Alternaria (0.3-1%)
- Cladosporium (0.1-1%)
- Aspergillus (0.3-3%)
- Penicillium (0-0.6%)
- Over 40 fungal genera with possible yeast form
STRUCTURE/FUNCTION OF BIOLOGICALLY ACTIVE CONSTITUENTS OF MICROBIOME MATTERS FOR HEALTH

3-Hydroxy Fatty Acid (3-OHFA) levels in House Dust and Asthma at age 7 (HAA Boston Area Birth Cohort)

<table>
<thead>
<tr>
<th>3-OHFA (Lipid A Constituent) Exposure in House Dust</th>
<th>Current Asthma* Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Mid Chain” Fatty Acids (C12:0-C14:0) ≥ Median</td>
<td>0.38 (0.15 to 0.99)</td>
</tr>
<tr>
<td>“Long Chain” Fatty Acids (C16:0-C18:0) ≥ Median</td>
<td>1.24 (0.52 to 3.0)</td>
</tr>
</tbody>
</table>

*Models adjusted for age, race, sex, maternal asthma

Sordillo et al, CEA (2010)
STRONGEST PREDICTORS OF INFANT GUT MICROBIOME ARE NOT BUILT ENVIRONMENT CHARACTERISTICS

<table>
<thead>
<tr>
<th>VDAART Cohort (N=325)</th>
<th>Shannon Diversity Infant Gut Microbiome</th>
<th>β coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian Race (vs. AA)</td>
<td></td>
<td>-0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-section Delivery</td>
<td></td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Breast Feeding</td>
<td></td>
<td>-0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at Fecal Flora Sampling (weeks)</td>
<td></td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Dog ownership</td>
<td></td>
<td>-0.03</td>
<td>0.60</td>
</tr>
<tr>
<td>Cat ownership</td>
<td></td>
<td>-0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>Water damage in home</td>
<td></td>
<td>-0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>Mold/Mildew in home</td>
<td></td>
<td>0.0002</td>
<td>0.40</td>
</tr>
<tr>
<td>Day care attendance</td>
<td></td>
<td>-0.05</td>
<td>0.80</td>
</tr>
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Model $R^2=22\%$

Shannon Diversity Index
- Univariate models showed some relationship for pets and gut diversity, but effects confounded by race

Taxonomic Profiles (16S) of the Infant Gut in VDAART
- No association between home characteristics and genus level taxa ($q > 0.05$) in a multivariate analysis (MaAsLin)
MINIMAL OVERLAP BETWEEN MICROBIOTA IN HOME AND INFANT GUT

Dust and Stool communities are distinct and do not cluster by pet ownership

BUT... Some taxonomic overlap detected:

14 OTUs overlapped in 20 paired house dust and infant stool samples

*Bifidobacterium, Streptococcus, Escherichia, Lachnospiraceae*

Stool → Dust or Dust → Stool???

(CHILD STUDY RESULTS; KONYA ET AL, ENVIRONMENTAL RESEARCH 131 (2014) 25-30)
## ANALYSIS METHODS FOR MICROBIOME COMPARISONS

| Built Environment Characteristics/Demographics → MOBE |  |
|---|---|---|
| **MaAsLin**<br>(Huttenhower Lab) | **Multivariate Analysis Technique** | Considers all taxa simultaneously as outcomes<br>Adjusts for Covariates |

| MOBE → Health outcomes |  |
|---|---|---|
| **LeFSe**<br>(Huttenhower Lab) | **Linear Discriminant Analysis Technique** | Identifies Microbiome features that discriminate between phenotypes<br>Utilizes any feature type |
| **PERMANOVA**<br>(R package/Adonis) | **Permutational Multivariate Analysis of Variance** | Identifies overall differences between communities<br>Adjust for covariates |

| Beyond rRNA gene sequencing using 16S data |  |
|---|---|---|
| **Oligotyping**<br>(Eren et al, PNAS 2014) | **Technique to uncover/decompose concealed diversity in OTUs** | Oligotypes may relate to functionally different microbial taxa |
| **PICRUSt**<br>(Huttenhower lab) | **Computational approach to predicting the functional metagenome** | Relative abundance of functional metabolic pathways (potential) |
INFANT GUT MICROBIOME FEATURES THAT DISCRIMINATE LOWER RESPIRATORY TRACT INFECTION (LRI) VS. NO LRI IN THE 1\textsuperscript{ST} YEAR OF LIFE

Adjusted Logistic Regression

LRI

OR=1.5 (95% CI 1.2 to 1.9) for IQR (1%) increase in \textit{Cronobacter} (Enterobacteriaceae)

OR= 1.4 (95% CI 1.37 to 1.44) for IQR increase (14%) in \textit{Escherichia/Shigella}

No LRI

OR=0.11, 95% CI 0.02 to 0.85 for detectable \textit{Peptophilus} (Clostridiales)

OR=0.2, 95% CI 0.04 to 0.87 for detectable \textit{Kluyvera} (Enterobacteriaceae)

*Adjusted for gender, c-section, race, breastfeeding, Vit D treatment group
Microbiome of Built Environment

Building Characteristics / Human Behaviors

Human Microbiome

Compartment(s)?

Microbiome of Built Environment


Human Health Outcomes

Opportunities for intervention? Health disparities research?
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