The Environmental and Infant Gut Microbiomes and Allergic Disorders: Human Studies

New Perspectives: Addressing the Asthma & Allergy Epidemics

Detroit, October 2015

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Disclosures
Christine C Johnson, PhD

No relevant financial relationships.

Personal financial interests in commercial entities that are relevant to my presentation: None

No discussion of off label drug use

Research Support: National Institutes of Health, Fund for Henry Ford Hospital

Legal Fees: None

Gifts: None

Other potential conflicts: None
Risk of Hay Fever Inversely Related to Number of Older Siblings

The Beginning of the “Hygiene Hypothesis”

Strachan, BMJ 1989; 299: 1259-60
Hygiene Factors

- Decreased family size
- Increased standard of living
- Suburbanization
- Less exposure to animals
- More immunizations
- More antibiotics use
Pregnant Mothers Living on Bavarian Farms (ALEX Study, n=901)

Farming, Bavarian Style
Pets in First Year of Life Inversely Associated with Allergic Sensitivity at age 6 yrs

Ownby et al. JAMA 2002
Evolution of the HYGIENE HYPOTHESIS

1989: DECREASED INFECTIONS?

2000: DECREASED BACTERIA EXPOSURE?

2007: CULTURE INDEPENDENT TECHNOLOGY

THE MICROBIAL DYSBIOSIS HYPOTHESIS
Microbial Community Composition In Home Household Characteristics PET Prenatal Immune Status Baby's Genotype, Season, SES, Delivery Mode, URIs, Antibiotics, Diet, Activity, Pets, Other Children, Pollutants, Stress Baby/Child’s Gut Microbial Community Composition Early Immune Response & Development Persistent Immune Response Phenotype Allergic Asthma
Questions...

- What environmental and social characteristics are related to the environmental microbiome?

- What social and environmental characteristics are related to the infant gut microbiome?

- How does the infant gut microbiome relate to atopic conditions?
**Wayne County Health, Environment, Allergy & Asthma Longitudinal Study (WHEALS) Birth Cohort**

- Pregnant mothers recruited 2003-2007, from Henry Ford Health System OB clinics in metropolitan Detroit Michigan USA (urban/suburban)

- Racially diverse (50% minority)

- Diverse socio-economic status

- Population-based (n=1258)

- Conducted interviews with mothers at prenatal and approximately 1 month (neonate) and 6 month (infant) home visits

- Dust and Stool samples collected at same home visits
The Indoor Microbiome: What does the dust tell us?
Are Babies exposed to House Dust?

- Hand-to-mouth activity in all children
- Well studied by toxicologists
- Average dust ingestion is 30–100 mg/day (20 – 70 million bacteria) for children 6 months – 11 yrs of age.

*U.S. EPA. Child Specific Exposure Factors Handbook 2008*
Bacterial Communities* in House Dust from Dog vs No-Pet Households

*measured by PhyloChip

Fujimura KE, JACI 2010;126:410-412
Atopic Wheezers Associated with Lowest Allergen & Bacterial Exposures In House Dust, URECA Cohort, age 3 yrs

Lynch, JACI 2014
How Do Environment and Social Factors affect the Environmental Microbiome?

- Studies show that dogs, cats and number of children affect the microbiome composition of the home.

- WHEALS 1 month and 6 month dust samples being measured

- Ongoing “Dog Adoption” Study

- Ongoing study funded by Sloan Foundation measuring home characteristics and microbial and fungal content of dust
The Infant’s Microbiome: What Do the Stools Tell Us?
Analytic Sample

• Drawn from WHEALS
• 298 stool samples met inclusion criteria and had sufficient DNA load for sequencing
  ▫ 1 month study visit: N=130, Median=35 days, IQR=17 days
  ▫ 6 month study visit: N=168, Median=201 days, IQR=37 days
• MAAP Sample representative of WHEALS in terms of:
  ▫ Race
  ▫ Pet ownership
  ▫ Gender
  ▫ Family history of allergic disease
  ▫ Mode of Delivery
• Tended to be higher income participants
Data Collection and Measurement

Environmental, Social & Clinical
- **What:** Maternal, Birth, and Household Early Life Characteristics
- **When:** Prenatal Period, 1 (neonatal) and 6 (infant) Month Home Study Visits
- **How:** Questionnaires, Chart Abstraction, Dust Samples, Medical Records, Clinical Exams

Microbiome
- **What:** Infant Gut Microbiome
- **When:** Neonatal and Infant Home Study Visit
- **How:** Illumina MiSeq Sequencing platform -tag sequencing of the 16S rRNA gene (v4 region) to identify bacteria present (Operational Taxonomic Units or OTUs)
Analytic Approach

- Differences in stool bacterial indices of richness, evenness and diversity: Wilcoxon Rank Sum tests

- PERMANOVA: tests compositional differences in microbiomes using Operational Taxonomic Units (OTUs)

- Principal Coordinates Analysis: PCoA – graphical depiction of distances (weighted or unweighted Unifrac) between subjects based on multidimensional data (thousands of OTUs):
  
  visual display of bacterial community composition differences by subject

- Dirichlet Mixture Model: identifies distinct microbiome profiles based on OTUs
Bacterial Family Relative Abundance by Sample Time in WHEALS Children
Baby Stool Bacterial Family Compositional Differences: by Mode of Delivery

- 1 month stools
- p-value < 0.001
- $R^2 = 1.9\%$
Pet-Keeping associated with bacterial Phyla Composition at 1 month visit; p-value=0.026
Bacterial Community Composition Differs by Breastfeeding

Both p-values $<0.001$
How does the Infant’s Microbiome relate to Disease Outcomes?
First Year Gut Microbiome Stratifies into Four Distinct Enterotypes

Dirichlet Mixture Model to statistically define infant sub-populations based on microbiome composition

- Bifidobacteriaceae - dominated
- Enterobacteriaceae - dominated
- Lachnospiraceae - dominated
- Bacteroidaceae/Bifidobacteriaceae /Lachnospiraceae – Co-Dominated

4.5% of the variation explained (p<0.001)
Prevalence of Sensitization at 2 yrs (allergen sIgE >0.35) for 10 Allergens within each Latent Class

Havstad et al. 2014

Predominantly Multi-sensitized group (PM group)
Infants with Co-Dominant Neonatal Enterotype had Higher Risk of Developing Multi-Sensitization

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NEONATES</th>
<th>Risk Ratio (95% CI)</th>
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<tbody>
<tr>
<td>Multiple sensitization</td>
<td>B to L</td>
<td>p-value</td>
</tr>
<tr>
<td>1.43 (0.73-2.81)</td>
<td>1.02 (0.59-1.75)</td>
<td>0.94</td>
</tr>
<tr>
<td>2.94 (1.42-6.09)</td>
<td>2.06 (1.01-4.19)</td>
<td>0.034</td>
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Key

E = Enterobacteriaceae  B = Bifidobacteriaceae  C = Co-Dominant  L = Lachnospiraceae
The development of allergy and asthma is:

- mainly influenced by gut *microbes* to which a child is exposed in the *first year* of life
- the composition of these gut microbes is determined by maternal and environmental factors.
Our Cities: Microbial Deserts?
## Acknowledgments

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<tr>
<th>MAAP Investigators</th>
<th>University of California-San Francisco</th>
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**Funding**

National Institutes of Health P01AI089473; R01A150681; R21AI080066; R01AI59415; R21AI 69271; R01AI61774; R01HL113010; R21ES022321

Sloan Foundation; FHFH

**Participants**

We thank the families and children who have participated in the WHEALS birth cohort and other P01 and HFHS birth cohort studies.
QUESTIONS?
Supplementary Figure 1: MAAP Sample Selection

Enrolled in WHEALS
N = 1258

Excluded prior to Year 2 visit
N = 255
- Deceased (N=7)
- Left study area (N=56)
- Refusals (N=66)
- Dropped for non-compliance (N=126)

Eligible for Year 2 visit
N = 1003

No paired stool/dust available (N=459)

Paired stool/dust available
N=544

Missing atopic status (N=236)

Eligible for MAAP-P1
N=308*

*N=298 stool samples with sufficient DNA for sequencing
Figure 2. Within-family richness by stool sample collection time. Figure displays all families with a significant trend (FDR adjusted p-value<0.05) and is ordered by significance (e.g., Lachnospiraceae is most significant). Color indicates direction of association.
MAAP – Stool Samples

Selection criteria:

• Needed to have 2 year outcome data

• Needed a “paired” dust and stool sample available in our repository for microbiome analyses at either the 1 month or 6 month visit

• Family still in study so eligible for future visits

N=308 stool/dust pairs sent to Univ California-San Francisco laboratory (Susan Lynch’s lab) for processing
Conclusions

• Breastfeeding may protect against colonization of specific Lachnospiraceae bacteria at 1 month of age
  ▫ Associated with increased risk of allergic-like response to pets at age 4
  ▫ Demonstrated significant functional differences that may contribute to differential immune response

• Lachnospiraceae: common adult gut colonizers
  ▫ Newborns (1%) → Infants (10%) → Adults (17%)
  ▫ In terms of gut microbiome, does breastfeeding prevent a premature shift to adulthood?
Compositional Differences by Allergic-Like Response to Pets

1 Month Visit:
• p-value = 0.023

6 Month Visit:
• p-value = 0.60
Clustering of factors univariately or multivariately associated with compositional differences in the neonatal and infant gut microbiome. Uses Unweighted UniFrac to define between-factor dissimilarity. Factors are colored if they were included in a multivariate model: 1.) Blue = retained in unweighted UniFrac model only, 2.) Green = retained in weighted UniFrac model only, and 3.) Red = retained in both models.
Significant Associations between Dust and Stool Mycobiomes in Late Infancy

- Mantel test using Canberra dissimilarity measures
- Significant positive association between the fungal dust and fungal stool communities in infancy
  Implies that samples with similar fungal dust microbiome also have similar fungal stool microbiome composition
- Demonstrates a significant link between household fungal exposure and the infant gut mycobiome
Univariate gut microbiome compositional analyses.

Only displays factors significantly associated with composition (p value < 0.05)
Three Microbe Defined Social Cultural Clusters

- **Underlying/Latent groups**
  - MSC1: 48% (n=609)
  - MSC2: 38% (n=474)
  - MSC3: 14% (n=175)

*Microbiome associated factors*
- Black race
- Married
- C-section
- Pet(s)
- ETS
- Breastfeeding

*Underlying/Latent groups*
- **Black Race**
  - MSC1: 48% (n=609)
  - MSC2: 38% (n=474)
  - MSC3: 14% (n=175)

- **Indoor Pet(s)**
  - MSC1: 48% (n=609)
  - MSC2: 38% (n=474)
  - MSC3: 14% (n=175)

- **Married**
  - MSC1: 48% (n=609)
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- **ETS**
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  - MSC3: 14% (n=175)

- **C-Section Delivery**
  - MSC1: 48% (n=609)
  - MSC2: 38% (n=474)
  - MSC3: 14% (n=175)

- **Breastfeeding**
  - MSC1: 48% (n=609)
  - MSC2: 38% (n=474)
  - MSC3: 14% (n=175)