Effects of formula supplementation on the composition of the infant microbiome

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The human microbiome

- The “forgotten organ”
- Co-evolved with humans
- 100 trillion organisms in the intestine
- 1000 species Qin J et al. 2010
- 10 microbes for every human cell
- 100 microbial gene for every human gene
- Innate and adaptive immunity evolved to require microbial interactions during development Lee YK et al. 2010, Chow et al. 2010
Consequences for health

• Out-competing pathogens
• Conferring resistance to infection (Gill et. al. 2012, Britton and Young 2012, Olszak et. al. 2012)
• Reducing susceptibility to inflammatory and metabolic disorders (Frank et. al. 2011, Nieuwdorp et. al. 2014)
  – Microbial diversity in the *first weeks of life* related to allergy at school age (Wang et al. 2008; Bisgaard et al. 2011)
The microbiome at birth

- Recent evidence points to some exposure of fetus to microbes through placenta, umbilical cord and/or amniotic fluid (Jiménez et al. 2007, Aagaard et al. 2014)

- Major colonization event at birth
  - Vaginal microbiome shifts during pregnancy to become dominated by *Lactobacillus* (Aagaard et al. 2012)
  - Human milk oligosaccharides promote the proliferation of *Bifidobacterium infantis* in infant intestinal tract (Coppa et al. 2004)
Delivery mode and feeding

Feeding method (breast milk vs formula) and delivery mode (vaginal vs. C-section) are the most commonly found correlates of microbiome composition in young infants.

Maria G. Dominguez-Bello et al. 2010

Tannock et. al. 2013

Azad et. al. 2013
New Hampshire Birth Cohort Study

- 1500 mothers who used a private well enlisted during 2nd trimester of pregnancy
- Infant stool collection:
  - Birth
  - Infant stool collection:
    - 6 week stool
    - 4 month stool
    - 8 month stool
    - 1 year stool
    - 6 week stool
    - 1 year stool

16S rRNA gene sequencing and whole genome metagenomic sequencing
Untargeted metabolomic profiling

N=300
N=50
N=300
Exposure data

• Delivery mode abstracted from delivery medical record

• Infant feeding over first 6 weeks of life ascertained by follow-up questionnaire
  – Exclusive breast feeding
  – Partial breast feeding (‘combination feeding’)
  – Exclusive formula feeding

• Infant urinary arsenic concentration measured at time of 6 week stool collection
Delivery mode

![Graph showing the association between delivery mode and bacterial species. Red circles represent vaginal delivery, black squares represent C-section delivery.](image)

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Combination feeding vs. ejection feeding vs. exclusive formula feeding association with exclusive breast feeding association with exclusive formula feeding

Lactococcus

Phascolarctobacterium

Exc

Sutterella

Escherichia

Bacteroides

Atopobium acetigenium

Anaerobius baezii

Sporolactobacillus

Propionibacterium

Actinomyces naeslundii

Coronella

Staphylococcus

Finegoldia

Peptostreptococcus

Sporacetigenium

Haemophilus

Between-group comparisons

Arsenic and the microbiota

- Mouse model: 10 ppm As for 4 weeks in drinking water  
  Lu et al. 2014

- As significantly perturbs the gut microbiome composition

- Metabolomics revealed metabolites perturbed
NHBCS arsenic exposure model

Carignan, Karagas et al. 2015
Infant Urinary Arsenic

Significant association between microbiome composition and
\[ \ln(\text{infant urinary arsenic concentration}) \] \( p = 0.006 \)

Among exclusively breast fed infants \( p = 0.38 \)
Among formula fed infants \( p = 0.009 \)
Maternal diet

- Maternal nutritional and immune status may be influenced by diet, which may also influence fetal development and subsequent host response to microbial population at and after birth.
- During normal vaginal delivery, the infant is exposed to both the maternal vaginal and gut microbiomes.
- Breastmilk includes nutrients, microbes, and immune factors that may be influenced by the maternal diet.

Study Population
Acknowledgements
Microbial Community Structure Results
Microbial Metagenome Results

• Sample Collection at 6 weeks postpartum

Prenatal Period
Delivery
Postnatal Period

Methods

Prenatal Food Frequency Questionnaire

Abstract

In Infants Born by Cesarean Section
Biospecimens

忧虑
PCoA of Generalized UniFrac Distances Colored by Fruit Consumption Tertiles in Infants Born Vaginally

PCoA of Generalized UniFrac Distances Colored by Dairy Consumption Tertiles in Infants Born by Cesarean Section

- 1st Tertile
- 2nd Tertile
- 3rd Tertile
- Continuous crude p-value = .04 *
- Continuous adjusted p-value = .03 *
- Tertile crude p-value = .10
- Tertile adjusted p-value = .14

- 1st Tertile
- 2nd Tertile
- 3rd Tertile
- Continuous crude p-value = .04 *
- Continuous adjusted p-value = .04
- Tertile crude p-value = .007 **
- Tertile adjusted p-value = .001 ***
Future plans

• High between-subject variation—need for longitudinal sampling
• Identify other factors important in shaping stool microbiome—likely a wide range
• Metabolomics to understand functional associations
• Exposure → microbiota → health outcomes
• Microbe-microbe interactions
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