CHILD Microbiome: Celebrating the Past & Planning the Future

Meghan Azad, PhD
Shirin Moossavi, MD PhD(c)
Kelsey Fehr, MSc

Children’s Hospital Research Institute of Manitoba
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World Microbiome Day (June 27)

- Microorganisms include bacteria, fungi, viruses, archea & protists
- Found everywhere in and on plants, animals, water, soil, food & humans
- They live in communities: microbiome
- Affect human, animal & environmental health!
- 34 events, 2 in Canada

#MindYourMicrobes and prevent Antibiotic Resistance!
Outline

- Brief CHILD Study overview
- Early microbiome development & gut-lung axis
- Findings from CHILD so far
- Future plans for microbiome research in CHILD
Brief Overview of CHILD
The Canadian Healthy Infant Longitudinal Development (CHILD) Study

How do genes and the environment influence child health and development?

$50M Invested
500,000 Samples: Blood, Urine, Stool, Nasal Swabs, Dust, Breast Milk
200,000 Questionnaires
3600 Families
40+ Researchers
20+ Disciplines
8(+) Years Follow-Up
93% Retention

www.canadianchildstudy.ca
CHILD Cohort Study

PRENATAL EXPOSURES

Maternal Nutrition

POSTNATAL EXPOSURES & OUTCOMES

Home Environment (Indoor, outdoor), Parents & Child Characteristics

Child Nutrition & Physical Activity

Viral Infections & Health Status

Prenatal 2009-2012

Birth

3 month

12 month

3 year

5 year

8 year 2018-2020

Clinic Visit

Hospital Visit

Home Visit

Clinic Visit

Clinic Visit

Clinic Visit

Clinical Visit

Breastmilk

Home Dust

Infant feces

Infant Nasal Swab

Infant Urine

Infant Blood

16S rRNA sequencing

ITS2 rRNA sequencing

*Shotgun metagenomics

*Metabolomics

Rich Multi-Dimensional Dataset
Microbiome Research in CHILD

Anita Kozyrskyj
Co-PI, SyMBIOTA & National Microbiome Core
GUT MICROBIOME

Sonia Anand
Canada Research Chair
Ethnic Diversity and Cardiovascular Disease
ETHNICITY & MICROBIOME

Michael Surette
Canada Research Chair in Interdisciplinary Microbiome Research
NASAL MICROBIOME CULTURE-ENRICHED MOLECULAR PROFILING

Stuart Turvey
Canada Research Chair in Precision Pediatrics
GUT MICROBIOME & MYCOBIOME HUMAN & ANIMAL STUDIES

Brett Finlay
Officer of the Order of Canada
GUT MICROBIOME & MYCOBIOME HUMAN & ANIMAL STUDIES

Meghan Azad
Canada Research Chair in Developmental Origin of Chronic Diseases
MILK MICROBIOME
Early Microbiome & Immune Development
Concurrent infant microbiome/immune development

Intra-uterus exposure to bacteria (?) or bacterial products

Initial colonisation depending on birth mode: surgical vs. vaginal

Acquiring microbes depending on feeding and home environment

Transition to adult-like stable microbiota

Gut-Lung axis

**Lung**
- Mucosal immune cell types & function
- Susceptibility to viral infection
- Hyperresponsiveness to allergens

**Gut**
- Immunogenicity & induction of IgA response
- Immune cell differentiation
- Differential abundance of different cell types
- Antibody diversification
- Microbial-derived small molecules (metabolites, MAMPs, virulence factors)
- Immune tolerance

Sources & determinants of infant microbiota development

Determinants of infant microbiota composition

Completed
• Milk microbiome (bacteria & fungi)
• Gut microbiome (bacteria)

Ongoing
• Gut microbiome (fungi)
• Nasal microbiome (bacteria)

• Maternal & household microbiota
• Nutrition
• Infant characteristics
Microbiome Highlights from CHILD
Breastfeeding, birth mode & gut microbiome

Birth mode
Pioneering microbial species
First colonizers of infant gut

Infant feeding
Continual delivery of microbes
Provision of human milk oligosaccharides
Conferring passive immunity

Influence on infant microbiome development trajectory (Gut & Nasal)

Azad et al. (2013) CMAJ; 185: 385-394
Breastfeeding & infant gut microbiota

Early exclusive breastfeeding reduces gut microbiome diversity

Early exclusive breastfeeding at 3 months influences the composition of gut microbiota:
- Proteobacteria & Actinobacteria
- Firmicutes

Forbes et al. (2018) JAMA Pediatr;172:e181161
Early-life antibiotics & gut microbiome

Birth mode
- Pioneering microbial species
- First colonizers of infant gut

Infant feeding
- Continual delivery of microbes
- Provision of human milk oligosaccharides
- Conferring passive immunity

Early life antibiotics
- Disturbance in the microbial community
- Elimination of susceptible commensal bacteria
- Survival/emergence of resistant bacteria
- Change in intestinal epithelial biology
- Absorption into peripheral circulation

Influence on infant microbiome development trajectory (Gut & Nasal)
Birth Mode, Intrapartum Antibiotics, Breastfeeding & Microbiota: 3 Months

**Microbiota at 3 months**

<table>
<thead>
<tr>
<th>Phyla:</th>
<th>Bacteroidetes</th>
<th>Proteobacteria</th>
<th>Verrucomicrobia</th>
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</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
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</tbody>
</table>

Azad et al. (2015). BJOG
Birth Mode, Intrapartum Antibiotics, Breastfeeding & Microbiota: 1 year

(Azad et al. BJOG 2015)
Dust microbiota & infant gut microbiota

Konya et al. (2014) Environmental Res; 131: 25–30
Household disinfectants & infant gut microbiota

Ethnicity and gut microbiome

SA: South Asian
C: Caucasian

No Major difference detected

Genetics, Diet, Cultural Practices

Gut Microbiota

Maternal asthma and infant gut microbiota

Male

Female

Milk microbiota influenced by maternal & infant characteristics and bottle feeding

Adapted from Moossavi et al. (2019) Cell Host Microbe; 25: 324-335

Moossavi & Azad (submitted)
Does milk microbiota shape the infant gut microbiota?

- Does milk contain microbiota that transfers to the infant gut during breastfeeding?
- How important is milk microbiota to infant gut microbiota composition?
- Challenge: common sources of microbiota to milk and infant gut (environment, infant oral cavity)

Conclusion: Microbiota are shared more commonly between a mother and her own infant.

Fehr et al., (Unpublished)
Asthma & Allergy

Pathophysiology

- A complex disease: Interaction of genes, the environment and immune system

Role of microbiota

- Gut microbiota
  - Modulate the development of immunotolerance
  - Mucosal immune cell population differentiation
  - Microbial derived small molecules (e.g. metabolites)
  - Migration of differentiated immune cells

- Upper respiratory tract microbiota
  - Predispose or protect against viral/bacterial upper respiratory infections
  - Effects on lung mucosal immunity

Microbiome modulating early life events can predispose or protect against disease

Infant gut microbiota & food sensitization

Azad et al. (2015) Clinical & Experimental Allergy, 45 : 632–643
Gut microbiota at 3 mos & atopic wheeze at 1 yr

Experimental validation in animal model

Gut microbiota Transfer from a 3m infant with atopic wheeze
Conventional
Germfree
Germfree

Colonization with FLVR
Conventional
Germfree
Germfree

F1 generation
Conventional
Germfree
Germfree

Ovalbumin sensitization

Total immune cell count
Neutrophils and Lymphocytes
Histopathology damage

Future Directions: Microbiome in CHILD
Future Directions

1. Understanding the pathophysiological heterogeneity of diseases.
2. How prenatal immune development influences infant microbiome succession and maturation?
3. How early life events modulate infant microbiome function?
4. How does host genetics interact with environmental factors to affect the infant microbiome?
5. How are different infant microbial communities (gut & lung) mechanistically contributing to
   ● Atopy?
   ● Atopic asthma?
   ● Obesity?
   ● Obesity-associated asthma
   ● Other diseases
6. Translational research to identify preventive and/or therapeutic targets
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CHILD Cohort Study

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SyMBIOTA: Anita Kozyrskyj, James Scott & Team

Canadian Healthy Infant Longitudinal Development Study

Canadian Institutes of Health Research

Allergen

DEVOTION: Developmental Origins of Chronic Diseases in Children Network

Breathe

Canada Research Chairs

Research Manitoba

Manitoba Health Research

Healthy Child Manitoba

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Canada’s Medical Research Network: Putting Research into Practice