SHAPING THE GUT MICROBIOME DURING INFANCY

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From growing microbes to ............
........identifying them by their DNA

OTU 1

TaxonGap

Naser et al., 2007

OTU 2

Lachnospiraceae (Family)

Unknown: OTU34

Streptococcus

Escherichia

x80 million

---

OTU

Naser et al., 2007

Lachnospiraceae (Family)

Unknown: OTU34
More friends in the gut than you think
IMPACT OF EARLY LIFE EXPOSURES ON INFANT GUT MICROBIOTA

by Mon Tun
My session objectives are to:

- Introduce the SyMBIOTA (Synergy in Microbiota) research program & the CHILD (Canadian Healthy Infant Longitudinal Development) birth cohort

- Present findings on the maturation of the gut microbiome over the 1st year of life and how this affected by early life exposures
Taxonomy 101 (Biological Classification)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Animalia</th>
<th>Bacteria</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Chordata</td>
<td>Bacteroidetes</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>Class</td>
<td>Mammalia</td>
<td>Bacteroidia</td>
<td>Clostridia</td>
</tr>
<tr>
<td>Order</td>
<td>Primates</td>
<td>Bacteroidales</td>
<td>Clostridiales</td>
</tr>
<tr>
<td>Family</td>
<td>Hominidae</td>
<td>Bacteroidaceae</td>
<td>Clostridaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Homo</td>
<td>Bacteroides</td>
<td>Clostridium</td>
</tr>
<tr>
<td>Species</td>
<td>Homo sapiens</td>
<td>Bacteroides</td>
<td>Clostridium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fragilis</td>
<td>difficile</td>
</tr>
</tbody>
</table>
The Canadian Healthy Infant Longitudinal Development (CHILD) Study

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

N=3600

www.canadianchildstudy.ca
CHILD Study
HELP CHILDREN GROW UP HEALTHY

$30M Invested
500,000 Biological Samples Banked
200,000 Questionnaires Completed
3600 Families Participating
92% Retention at 1 year
40+ Senior Researchers
20+ Scientific Disciplines:

Air Quality | Infectious Disease | Physiology
Biostatistics | Molecular Biology | Population Health
Endocrinology | Neonatology | Psychology
Environmental Health | Neuroimmunology | Respirology
Epidemiology | Nutrition | Sociology
Genetics | Obstetrics | Toxicology
Immunology | Pediatrics | Microbiome

MILESTONES

- **JULY 2018**: 5 Year Visits Complete
- **JULY 2017**: 4 Year Qn Complete
- **JULY 2016**: 3 Year Visits Complete
- **JULY 2015**: Infant PFT Visits Complete
- **JULY 2014**: 1 Year Visits Complete
- **JULY 2013**: Home Visits Complete
- **JULY 2012**: Recruitment Complete

OUTCOMES

- **JULY 2018**: 5 Year Clinical Assessment
  - Asthma, Allergy, Sleep, Psychosocial, Environmental Outcomes
- **JULY 2017**: 3 Year Clinical Assessment
  - Sleep and Neurodevelopment Outcomes
- **JULY 2016**: Viral Outcomes
- **JULY 2015**: Infant PFT
  - Neurodevelopment Outcomes
  - Innate Immunity Outcomes
- **JULY 2014**: 1 Year Clinical Assessment
  - Neurodevelopment, Sleep, Blood, Psychosocial, Microbiome Outcomes
- **JULY 2013**: Birth, Prenatal and Environmental Outcomes
- **JULY 2012**: Baseline Demographics

www.canadianchildstudy.ca
Metadata and infant fecal samples from the Canadian Healthy Infant Longitudinal cohort (www.childstudy.ca)

www.allergen-nce.ca/Research/SyMBIOTA.html

CIHR CDN MICROBIOME INITIATIVE TEAM GRANT, $2.5 million/5 yr
GUT MICROBIOME MATURATION IN FULL TERM INFANTS

Canadian Healthy Infant Longitudinal Development (CHILD) cohort
Development of gut microbiota over the first years of life (Jakobsson al. Gut 2014; 63)

Swedish birth cohort A, vaginally born infants

LACTOBACILLI & OTHER LAB

LACHNOSPIRACEAE & OTHER BUTYRATE PRODUCERS

Mean relative abundance (%)
Mean relative abundance (%) of microbial phyla

CHILD COHORT

Development of gut microbiome during infancy
Development of gut microbiome during infancy
Development of gut microbiome during infancy

CHILD COHORT

nMDS 1

nMDS 2
Important Role of Pioneer Gut Microbes

- Days after birth, facultative anaerobes, LAB (lactic acid bacteria, eg. lactobacilli, streptococci) and Enterobacteriaceae create an anaerobic environment allowing strict anaerobes, such as bifidobacteria, Clostridium and Bacteroides species, to thrive.

- Early microbial composition determines future composition
  - Heavier colonization with Enterobacteriaceae within 3 days of birth = greater abundance of bifidobacteria 6 months later (Dogra et al. 2015)
Lactobacilli (of Firmicutes) decline in gut microbiota with advancing infant age

Percent colonization with Lactobacillus species

Martin et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLOS One 2016; 11
How you are born, what you are fed and your exposure to antibiotics shapes your microbiome as an infant !!!!!!

![Table showing microbial diversity across different factors such as breastfeeding, birth mode, type of floor, study location, maternal race, siblings, type of home, size of household, and antibiotic exposure.]

Figure is available in: Tun et al. Exposure to household furry pets influences the gut microbiota of infant at 3-4 months following various birth scenarios Microbiome (2017) 5:40
CESAREAN BIRTH & GUT DYSBIOSIS IN EARLY INFANCY

Canadian Healthy Infant Longitudinal Development (CHILD) cohort
Development of infant gut microbiota over the first years of life (Jakobsson al. Gut 2014; 63)

Swedish birth cohort
A, vaginally born infants  
B, cesarean born infants  
DELAYED Bacteroides colonization
Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months

Meghan B. Azad PhD, Theodore Konya MPH, Heather Maughan PhD, David S. Guttman PhD, Catherine J. Field PhD, Radha S. Chari MD, Malcolm R. Sears MB, Allan B. Becker MD, James A. Scott PhD, Anita L. Kozyrskyj PhD, on behalf of the CHILD Study Investigators

Researchers studied 24 babies and compared the bacteria found in baby poop samples collected when each infant was just 3 months old. They knew, prior to the study, that C-section deliveries could result in a higher risk of asthma, diabetes, cancer and even obesity, but they didn’t know how. Their recent work suggests that at least part of that risk may be due to the microbes forming inside baby.
Contributing emerging microbiome evidence to clinical practice


- Kozyrskyj AL, Tun HM, Bridgman SL. The Microbiome and Control of Weight Gain in: *Pediatric Obesity: Etiology, Pathogenesis & Treatment*, Freemark M (ed). Springer Publisher, USA, 2018
Chapter 4: Birth & Postnatal Interventions

- Chapter 4 is organized by 4 themes:
  - Birth mode (vaginal birth vs scheduled or emergency cesarean)
  - Intrapartum maternal antibiotic prophylaxis (IAP for GBS)
  - Extended hospitalization post birth
  - Postnatal infant IV and oral antibiotic treatment

- Chapter table organized by the same themes plus
  - Taxon category of major changes ie. *Enterobacteriaceae*, *Bacteriodaceae*,

Chapter 4: TAKE HOME MESSAGE

- All 4 main early life exposures during infancy
  - ↓ Bacteroidetes species (see Rutayisire E et al systematic review), required for the maintenance of the gut mucin barrier
  - ↓ bifidobacteria
  - ↑ staphylococci
  - ↑ Clostridium (difficile)
  - ↑ Proteobacterial species

- Emergency cesarean and infant antibiotic use
  - ↑ enterococci
IAP, CESAREAN BIRTH & INFANT GUT DYSBIOSIS AT 3 MONTHS OF AGE

Almost 40% of infants are exposed to an antibiotic by the time they are born.

Bacteroidaceae abundance (orange) is reduced with IAP and CS. Other ↑s typical of antibiotic resistance.

The special case of emergency CS; mothers and newborns were more likely to receive antibiotics.

<table>
<thead>
<tr>
<th></th>
<th>Maternal PP antibiotics</th>
<th>Newborn antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>no IAP, vaginal</td>
<td>18.9</td>
<td>0</td>
</tr>
<tr>
<td>IAP, vaginal</td>
<td>18.9</td>
<td>4.8</td>
</tr>
<tr>
<td>IAP, elect CS</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>IAP, emerg CS</td>
<td>42.9</td>
<td>20</td>
</tr>
</tbody>
</table>

p<0.0001
HOSPITALIZATION POST BIRTH & GUT DYSBIOSIS AT 3-4 MONTHS OF AGE

Canadian Healthy Infant Longitudinal Development (CHILD) cohort
Prolonged hospital stay after birth: ↓ Bacteroidaceae but ↑ Lachnospiraceae, Enterobacteriaceae
DEVIATION IN GUT MICROBIAL DEVELOPMENT IN LATER INFANCY WHEN NOT BORN VAGINALLY OR BREASTFED

Canadian Healthy Infant Longitudinal Development (CHILD) cohort
Few taxon ↓ or ↑ between 3 months - 1 year of age in infants not breastfed = higher risk for food sensitization

MODERATING EFFECT OF EARLY BREAST FEEDING IN CESAREAN DELIVERY
Short-chain fatty acid (SCFA) produced by gut microbes in infants breastfed vs not: Total SCFA ↓ ... BUT ↑ % acetate (Bridgman et al. Frontiers Nutr 2017;4)
SCFA, acetate and propionate, reduce risk for allergic asthma in animal models

SCFA metabolites produced by gut microbes affect leptin & other satiety hormones, gluconeogenesis and lipid storage: Kumari & Kozyrskyj. Obes Rev 2017; 18
Cesarean section leaves an imprint (↘ Bacteroidetes in orange) at 3 months independent of breastfeeding.
But in the absence of exclusive breastfeeding at 3 months, Bacteroidaceae persists after emergency CS.
Antibiotics

Maternal

Infant

Birth mode
Vaginal
Cesarean

Maternal gut, vaginal or placental microbial dybiosis
Leaky gut and/or placenta
Vertical transmission of maternal microbes
Epigenetic effects of microbial metabolites

Infant sex, ethnicity
Male-female
Caucasian-other

Altered Gut Microbiota & Immunity
Bacteroides
Enterobacteria
Staphylococcus
Bifidobacterium
Lactobacillus
Fecal IgA

B. Postnatal factors influencing infant gut microbiota

Prenatal diet and health

Birth mode
Vaginal
Cesarean

Antibiotics
Maternal
Infant

Infant diet
Breastfeeding status
Breast milk microbiota


Kozyrskyj AL et al. Fetal programming of overweight through the microbiome: boys are disproportionately affected. J Dev Orig Health Dis 2016; 7: 25-34
PRENATAL INFLUENCE OF MATERNAL OVERWEIGHT, STRESS & ASTHMA
Birth mode and gut Lachnospiraceae JOINTLY mediate the association between maternal and child overweight

![Diagram](image)

Bootstrap 95%CI for indirect effect \((a_1d_21b_2)\)

\[
[0.0001, 0.0156]
\]

\[
[-0.0003, 0.0095]
\]

\(P<0.1, \ *P<0.05, \ **P<0.01, \ ***P<0.001, \ ****P<0.0001\)

Tun et al. Roles of birth mode and infant gut microbiota in intergenerational transmission of overweight and obesity from mother to offspring. JAMA Pediatr
Fecal slgA at 3 months was lower in infants of mothers with distress during pregnancy and afterwards

Secretory Immunoglobulin A measured in infant fecal samples at 3 months. It is critical to:

- Our gut immune defense system
- Immune system maturation in infants
- Induction of **oral tolerance** to food
- Reduced risk of **allergic diseases**

Breast Milk

Especially among infants not breastfed but also seen in breastfed infants

Independent of breastfeeding, ↓ fecal abundance of Lactobacillaceae at 3 months in **male** infants born to women with **asthma during pregnancy** (at risk for future asthma & overweight)

Koleva et al. Sex-specific impact of asthma during pregnancy on infant gut microbiota. Eur Resp J 2017;50
↑ fecal abundance of Bacteroidaceae at 3 months in female infants born to women with asthma during pregnancy (at risk for future asthma & overweight)

Koleva et al. Sex-specific impact of asthma during pregnancy on infant gut microbiota. Eur Resp J 2017;50
BACTEROIDETES + ENTEROBACTERIA = FOOD SENSITIZATION


Canadian Healthy Infant Longitudinal Development (CHILD) cohort
Food sensitization in infants is a 2-fold risk for asthma & allergic disease!

- Egg: 60%
- Peanut: 27%
- Milk: 13%
Species richness was significantly lower at 3 months but not 1 year in sensitized infants.
Fewer Bacteroidaceae/more Enterobacteria ($\uparrow$E/B) in food sensitized infants at 1 year

A) All Infants [N=166]
E/B ratio and low species richness associations with sensitization are independent of each other.

<table>
<thead>
<tr>
<th>Model Adjustments</th>
<th>Microbiota at 3 months</th>
<th>Microbiota at 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aOR (95% CI)</td>
<td>aOR (95% CI)</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mutual adjustment for microbiota only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/B Ratio (per quartile increase)</td>
<td>2.02 (1.07 - 3.80)*</td>
<td>4.14 (1.54 - 11.11)*</td>
</tr>
<tr>
<td>Chao1 Richness (per quartile increase)</td>
<td>0.45 (0.23 - 0.87)*</td>
<td>- -</td>
</tr>
<tr>
<td><strong>Adjusted for any antibiotic exposure before sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/B Ratio (per quartile increase)</td>
<td>2.13 (1.10 - 4.13)*</td>
<td>4.03 (1.50 - 10.82)*</td>
</tr>
<tr>
<td>Chao1 Richness (per quartile increase)</td>
<td>0.45 (0.23 - 0.87)*</td>
<td>- -</td>
</tr>
<tr>
<td><strong>Adjusted for caesarean delivery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/B Ratio (per quartile increase)</td>
<td>2.21 (1.13 - 4.31)*</td>
<td>4.35 (1.57 - 12.11)*</td>
</tr>
<tr>
<td>Chao1 Richness (per quartile increase)</td>
<td>0.42 (0.21 - 0.85)*</td>
<td>- -</td>
</tr>
<tr>
<td><strong>Adjusted for exclusive breastfeeding at 3 months</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/B Ratio (per quartile increase)</td>
<td>1.80 (0.93 - 3.50)</td>
<td>4.02 (1.48 - 10.88)*</td>
</tr>
<tr>
<td>Chao1 Richness (per quartile increase)</td>
<td>0.46 (0.24 - 0.89)*</td>
<td>- -</td>
</tr>
</tbody>
</table>
Acknowledgements

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LOOKING FOR NEW POSTDOCS
CHILD: Microbiota profiling methods

- Fecal samples at 3 months from 1000 full-term infants sequenced with MiSeq at V4
- Sequences clustered at 97% similarity against the Greengenes database (closed-picking) to identify Operational Taxonomic Units (OTUs)
- Rare OTUs (<0.0001 relative abundance) excluded
- Data were rarefied to 15,000 sequences per sample
- OTU relative abundance and diversity indices (Chao1, Shannon) were generated by the QIIME program