Early Life Microbiota Development and Host Health

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ONE
Microbes to Molecules

TWO
Diet & Microbes at the Extremes of Life

THREE
Brain- Gut Axis

FOUR
Host-Microbe Dialogue
Microbiome

IN NUMBERS

100 Trillion
symbiotic microbes live in and on every person and make up the human microbiota

95%
of our microbiota is located in the GI tract

150:1
The genes in your microbiome outnumber the genes in our genome by about 150 to one

The human body has more microbes than there are stars in the milky way

90%
of disease can be linked in some way back to the gut and health of the microbiome

5:1
Viruses:Bacteria in the gut microbiota

2.5
The number of times your body's microbes would circle the earth if positioned end to end

>10,000
Number of different microbial species that researchers have identified living in and on the human body

1.3X
You have more microbes than human cells

2kg
The gut microbiota can weigh up to 2Kg

Each individual has a unique gut microbiota, as personal as a fingerprint

The microbiome is more medically accessible and manipulable than the human genome

It is thought that
Intestinal Microbiota

*Complex and dynamic community*

- $10^{14}$ microorganisms/g contents
- 10 times more bacteria than human cells
- 100-fold more unique genes than our own genome
- Important functions:
  - Fermentation of food components
  - Production of SCFAs, vitamins, bioactives
  - Control of epithelial cell proliferation
  - Development of the immune system
  - Barrier effect
  - Production of antimicrobial substances/pathogen inhibition

Guarner *et al.*, 2011
The Gut Microbiome

Microbiome is redefining in terms of Microbiology, Nutrition and Human Health

Age, diet, host and antibiotics shape the microbiome but not precisely

How can we modulate the composition of the Microbiome?
Modulation of Intestinal Microbiota

“Open window of opportunity” (Fouhy et al., 2012)

- Importance of the correct establishment of intestinal microbiota
- Early postnatal period → constitutes *key moment*
- Modulation to the establish a healthy microbial profile

Early life: Seeting the immune balance for life

Kerperiem et al., 2012
Factors Affecting the Infant Gut Microbiota

Ventura et al., 2018
The first thousand days – intestinal microbiology of early life: establishing a symbiosis
Harm Wopereis\textsuperscript{1,2}, Raish Oozeer\textsuperscript{3}, Karen Knipping\textsuperscript{1}, Clara Belzer\textsuperscript{2} & Jan Knol\textsuperscript{1,2}

Microbiota

Immune System

Early microbiota

Allergy

Nutrition

Symbiosis
Maternal Vertical Transmission Affecting Early-life Microbiota Development

Shaopu Wang,1,2 C. Anthony Ryan,1,3 Patrick Boyaval,4 Eugene M. Dempsey,5,5 R. Paul Ross,1,6 and Catherine Stanton1,2,7

Vertical transfer of microbiota
....as we go through life....

C-section

Diet

Vaginal birth

Diet, Antibiotics, Illness

Formula

More complex
B. fragilis
E. coli
C. difficile

Breast

Bifidobacterium
Bacteroides

Stable core genome

Bacteroides
Clostridium
Ruminococcus
Eubacterium
Parabacteroides
Coprococcus

Dorea
Alistipes
Collinsella
Lachnospira
Roseburia
Faecalibacterium

Old age

Fusobacterium
Clostridium
Eubacterium
Facultative anaerobes

Bacteroides
Bifidobacterium
SCFA

After  Power et al, BNJ (2014) 111:387
Major Influence of Birth Mode on Early Microbiome

- CS dominated by skin-associated bacteria
- Infants born by elective CS have particularly low bacterial richness and diversity
- Escherichia Shigella and Bacteroides species were under-represented in CS infants
Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth

Shao et al. 2019 Nature
https://doi.org/10.1038/s41586-019-1560-1
Study Overview

Cohort

• 314 vaginal birth (23 intrapartum antibiotic prophylaxis)
• 282 caesarean section birth (all IAP)
• Subset of 175 mothers paired with 178 babies

Analysis

• Whole-genome shotgun metagenomic sequencing of 1,679 stool samples
• Targeted culture and whole-genome sequencing of pathogenic species present at >1% relative abundance in the infant or maternal stool
Results: Early-life faecal microbiota

- **Vaginal delivery**: *Bifidobacterium, Escherichia, Bacteroides* and *Parabacteroides* dominate throughout the neonatal period and into infancy.

- **C-section delivery**: Low relative abundance of *Bacteroides* spp., while *Enterococcus, Staphylococcus, Streptococcus, Klebsiella, Enterobacter* and *Clostridium* were 68.25% of the total faecal microbiota at day 4 (30.4% at day 21). Enriched populations of opportunistic pathogens persisted to infancy.

- No statistical difference in the prevalence of *Lactobacillus* spp. between birth modes.
Results: Transmission of maternal microbial strains

- Vertical transmission of maternal microbial strains occurred in 74.39% of vaginal babies and in 12.56% of c-section babies.
- Transmission of maternal *Bacteroides* spp., *Parabacteroides* spp., *E. coli* and *Bifidobacterium* spp. was most frequent in vaginally delivered babies vs c-section delivered babies.
- The neonatal period is a critical early window for maternal transmission of *Bacteroides*. 
1. How do perinatal factors such as birth mode and gestational age at birth affect microbiome development?

2. How does breast feeding affect microbiome composition—are there subsets that benefit more?

3. How to develop nutrition/microbiota interventions to improve early maturation of the microbiome?
INFANT GUT MICROBIOTA DEVELOPMENT FROM BIRTH

Infant groups (n = 50 per group):

- INFANTMET: Pre-term (<1500g or <35 weeks)
- INFANTMET: Caesarean section (full term)
- INFANTMET: Natural vaginal delivery (full term)
- MYNEWGUT: Caesarean section (full term)/Antibiotics

Week 1 Week 4 Week 8 Week 24 Year 1 Year 2-4

Age of infant

Urine
Saliva

Health questionnaire at year 1 and year 2
Table 1. Spearman’s rank coefficient comparing culturing techniques (log CFU g⁻¹ faeces) and MiSeq sequencing (relative number).

<table>
<thead>
<tr>
<th>Group</th>
<th>Bifidobacterium spp.</th>
<th>Lactobacillus spp.</th>
<th>No of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.24 (6.64 e⁻⁷)</td>
<td>0.21 (1.96 e⁻⁵)</td>
<td>199</td>
</tr>
</tbody>
</table>

*Spearman’s rank correlation coefficient with P-value in brackets

Infant Culture Collection
3,650 Bifidobacterium
3,500 Lactobacillus

Microbiome Diversity Increases over Time: INFANTMET

Microbiota development in first 24 weeks: INFANTMET Study

Evolution of microbiome: birth mode and gestational age

- **Full term Vaginal delivery**
- **Full term C-Section**
- **Preterm C-Section**

Weeks: 1, 4, 8, 24

Relative abundance

Phylum:
- Bacteroidetes
- Proteobacteria
- Firmicutes
- Actinobacteria

Other

$n = 250$ babies

Hill et al. Microbiome 2017
Microbiota diversity increases to 4 years

Fouhy & Watkins et al. (2019) Nature Communications
1. Genera in the bottom left corner of the map, including *Escherichia-Shigella* and *Enterobacter* present at greater abundances at year one.

2. Genera in the bottom right hand corner, including *Christensenellaceae spp.* and *Ruminococcaceae spp.* present at greater abundance at years two and four.

3. *Bifidobacterium* appeared at low abundance by year four.
Perinatal factors continue to affect the gut microbiome four years after birth

Discriminative taxa:
Year 1: Escherichia-Shigella and Bifidobacterium
Year 2: Lachnospiraceae_UCG008
Year 4: Christensenellaceae

At four years of age...

Alpha Diversity by Gestational age at birth

- Full term (>35 wk gestation)
- Preterm (<35 wk gestation)

Fouhy & Watkins et al. (2019) Nature Communications
Gestational age - imprint 4 years later

The microbiome has a memory of premature birth

Fouhy & Watkins et al. 2019 Nature Communications
Breastfeeding has greater effect on C-section babies

C-section

Naturally-delivered

5 genera were different in those breast fed for longer

Hill et al. Microbiome 2017
Structural composition of human milk oligosaccharides

- Over 200 structures identified
- 50-80% are fucosylated
- 10-20% are sialylated
- Most abundant: 2-fucosyllactose (2’FL)

Function of HMO
1. Antimicrobial and antiviral activity
2. Prebiotic effect
3. Mucosal barrier maturation
4. Effects on immune function
5. Modulation of pathogen recognition
Bifidobacteria abundance in the gut decrease with advancing age

- Gram +
- Strictly anaerobic
- Non-sporeforming
- Dominant genus in the breast-fed infant gut

61 (sub)species identified

B. longum
B. breve
B. bifidum

B. longum
B. pseudocatenulatum
B. catenulatum

B. longum
B. bifidum
B. adolescentis

Gut Bifidobacteria Populations in Human Health and Aging

Silvia Arboleya, Claire Watkins, Catherine Stanton, and R. Paul Ross
**Bifidobacterium longum** most dominant species in infants

- Highly competitive
- Numerous also in adults
**Bifidobacterium longum**

*B. longum subsp. infantis*

- 43 Kb gene cluster specialised for HMO utilisation
- 7 solute binding proteins (SBPs) for transport
- 4 internal glycosyl hydrolases (GH)
  - Sialidase
  - Fucosidase
  - N-acetyl-β-hexosaminidase
  - β-galactosidase

*Sela et al., 2008*

*B. longum subsp. longum*

- Specialised for plant-derived sugars
- A limited number have “fucosyllactose cluster”-correlates with growth on 2’FL and 3’FL

*Arboleya et al., 2018*
B. longum genome is highly variable

Gene families present at least once in all the examined genomes.

Gene families present in some of the genomes but not in all the examined genomes.

Arboleya et al (2018) BMC Genomics
Polybiotic – representing *B. longum* pangenome

- **Group A:** *B. longum* ssp. *infantis*
- **Group B:** *B. longum* new subspecies
- **Group C:** *B. longum* ssp. *suis*
- **Group D:** *B. longum* ssp. *longum*

Spread uniformly – good representation of this subspecies

Arboleya et al (2018) BMC Genomics
**Bifidobacterium bifidum**

- Specialised for host-derived sugars: HMO and mucin
- External GH degrade large, complex oligosaccharides to mono- and disaccharides
- Transport systems (PTS and ABC-type) to internalise disaccharides, eg. lactose, lacto-N-biose
- Sialic acid, fucose not utilised

*Milani et al., 2016, Turroni et al., 2010 Egan et al., 2014*
**Bifidobacterium breve**

- A number of distinct gene clusters to utilise HMO (DP ≤ 4)
  - Lacto-$N$-tetraose (LNT)
  - Lacto-$N$-neotetraose (LNnT)
  - Sialic acid
  - Fucose

- No external GH (for HMO metabolism)-sugars are consumed and degraded internally

- Can scavenge sialic acid, fucose released from extracellular GH activity of *B. bifidum*

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James et al., 2019
James et al., 2016
Egan et al., 2014
**Bifidobacteria: Strategies for HMO Utilisation**

**B. longum subsp. infantis**
- Internalises HMO through ABC-type transporters
- HMO degraded by internal glycosyl hydrolases

**B. bifidum**
- Large number of external glycosyl hydrolases
- Internalises lactose, lacto-N-biose, N-acetyllactosamine

**B. breve**
- Scavenges mono- and disaccharides liberated by *B. bifidum* eg. sialic acid
- Internalises short chain neutral HMO eg. LN(n)T

http://apc.ucc.ie
**Overall aims**

**Task 1:** To establish milk microbiome composition in milk, following full and preterm births, over lactation from birth to 6 months.

**Task 2:** To characterise the infant gut microbiota from birth to 6 months of age in breast-fed infants, through faecal analysis, and correlate with milk microbiome.

**Task 3:** To compare infant gut of breast-fed infants to that of a group of control infants who were exclusively formula fed.

**Task 4:** To generate a bank of human milk-derived strains for their characterisation and potential use as future probiotics for infant nutrition and health markets.

**Proof of concept**
- N = 10 mother-infant pairs
- Isolated viable Bif/Lac from milk
- Supported vertical transfer from mothers milk to infant gut
- Phyla – Proteobacteria, Firmicutes, Bacteroidetes
- Genera - *Pseudomonas, Staphylococcus, Streptococcus, Elizabethkingia, Variovorax, Bifidobacterium, Flavobacterium, Lactobacillus, Stenotrophomonas, Brevundimonas, Chryseobacterium* and *Enterobacter*
Bifidobacteria: one of 12 “core” genera in breast milk from weeks 1 to 12

Murphy et al., 2017

Isolation of a *B. breve* strain from breast milk and corresponding infant stool—

Vertical transfer between mother and infant
Macronutrients and micronutrients in breastmilk and infant formula

Breastmilk
- Water
- Carbohydrates
- Carboxylic acid
- Proteins
- Non-protein nitrogen
- Triglycerides
- Phospholipids
- Sphingolipids
- Sterols
- Vitamins
- Minerals
- Metal
- Growth factors
- Peptides
- Hormones
- Enzymes
- Antiproteases
- Antimicrobial factors

Artificial formula
- Water
- Carbohydrates
- Proteins
- Fats
- Minerals
- Vitamins
- Enzymes
- Amino acids
- Nucleotides

Ahern... & Stanton (2019). Annual Reviews in Food Science and Technology
Introduction of probiotics was associated with a reduced adjusted odds for ‘NEC or sepsis or death’ in exclusively breastmilk-fed infants.

The type of feeding seems to modify the effects of probiotics.

Conclusion

In order to reduce NEC and mortality in preterm infants, it is advisable to add routine prophylaxis with dual-strain probiotics to clinical practice in neonatal wards.
Effective Probiotic Dose?

- Preterm infants: (n=10) Stool samples collected for DNA extraction & **16S rRNA sequencing**.
- **Time points**: week 31, week 34, week 41, week 44 post-gestational age.
- *Bifidobacterium bifidum* (10⁹ CFU) and *Lactobacillus acidophilus* (10⁹ CFU/250mg tablet).
- **Three dosage groups**: Weekly, Bi-weekly, Daily.
- **Inclusion criteria**: < 32 weeks gestational age.

<table>
<thead>
<tr>
<th>(n= no. of infants)</th>
<th>Delivery Mode (n)</th>
<th>Feeding Regime (n)</th>
<th>Antibiotics Taken (n)</th>
<th>Incidences</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SVD</td>
<td>LSCS</td>
<td>EBM</td>
<td>Formula top up</td>
</tr>
<tr>
<td>Daily</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>BiWeekly</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Weekly</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>10</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>
Early life administration of antibiotics can lead to perturbation of optimal microbiota development.

Implications for long-term microbial diversity and consequent health?

**Aim:** To investigate the impact of the administration of a single dose of a commercially available probiotic, Infloran® (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*), on the gut microbiota of seven full-term infants who had received parenteral antibiotic treatment within the first 48 hours of life.

*(Watkins et al., 2016)*
Probiotic Dose Effects on Preterm Microbiota

% Relative abundance at phylum level

- **Other**
- **Proteobacteria**
- **Firmicutes**
- **Actinobacteria**

<table>
<thead>
<tr>
<th>Time</th>
<th>Post birth</th>
<th>Post gestational age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Week 1</td>
<td>31 weeks PGA</td>
</tr>
<tr>
<td>2</td>
<td>Week 4</td>
<td>34 weeks PGA</td>
</tr>
<tr>
<td>3</td>
<td>Week 11</td>
<td>41 weeks PGA</td>
</tr>
<tr>
<td>4</td>
<td>Week 14</td>
<td>44 weeks PGA</td>
</tr>
</tbody>
</table>

(Watkins et al., 2016)
Infloran-probiotic administration enhances gut microbiome diversity

Microbial composition at genus level

(Watkins et al., 2016)
The Swedish National Registry data were used to examine the association between Caesarean section (CS) and development of depression or anxiety.

All singleton, live births were identified which occurred from January 1st 1982 and December 31st 2001 (n=2,018,842).

A small increased risk of depression was found in offspring aged 10 years and older in assisted VD (HR 1.04, 95% CI 1.01, 1.08), elective CS (HR 1.04, 95% CI 1.01, 1.08) and emergency CS (HR 1.06, 95% CI 1.02, 1.10) when compared to unassisted VD.

A small increased risk of anxiety was found in offspring delivered via emergency CS only (HR 1.06, 95% CI 1.02, 1.11).
Trier Social Stress Test (TSST)

A) Key Procedural Stages:

1. Baseline Rest
   - Following baseline sample collections participant rests quietly alone in Room A

2. Task Instructions
   - "Imagine you have applied for your ideal job for which you must convince the committee members why you are the perfect candidate"

3. Stress Procedure
   - In Room B:
     - 3 Minute Preparation
     - 5 Minute Speech
     - 5 Minute Mental Arithmetic

4. Recovery Period
   - Participant returns to Room A for recovery and further measures are collected

B) Example Sampling Schedule:

<table>
<thead>
<tr>
<th>Baseline Rest &amp; Task Instructions</th>
<th>Stress Procedure</th>
<th>Recovery Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-30 minutes</td>
<td>t0</td>
<td>t+15</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>t+75</td>
</tr>
</tbody>
</table>

Immune Response (Plasma Cytokines)
HPA axis Response (Salivary cortisol)
Psychological Stress Response

TSST- Salivary Cortisol Response

C-section vs. Natural Born

- Natural Born (n=39)
- C-section (n=34)

Data unpublished
TSST: Psychological Stress Response

**a. TSST Positive Affect**
- Mean Score
- Natural Born
- C-Section

**b. TSST Negative Affect**
- Mean Score
- Natural Born
- C-Section

**c. TSST Psychological Stress**
- VAS (0-100%)
- Natural Born
- C-Section

**d. TSST Psychological Stress (AUCg)**
- VAS (0-100%)
- Natural Born
- C-Section

Data unpublished
Effects of exam stress

**State Anxiety**
- Natural Born
- C-Section

**Trait Anxiety**
- Natural Born
- C-Section

**Beck Depression Inventory**
- Natural Born
- C-Section

**Perceived Stress**
- Natural Born
- C-Section

Data unpublished
Cognitive Testing

- Paired Associates Learning (PAL)
- Stop Signal Reaction Time
- Intra/Extra Dimensional Set Shift (IED)
Effects of exam stress on cognitive function

**Visuospatial Memory**
- Natural Born
- C-Section

**Response Inhibition**
- Natural Born
- C-Section

**Attentional Set Shifting**
- Natural Born
- C-Section

**Reversal Learning**
- Natural Born
- C-Section

Data unpublished
Exposures to prenatal maternal depression.

Infection during gestation or early life.

Maternal nutrition and consequences.

THE GUT MICROBIOTA AS A REGULATOR OF THE STRESS RESPONSE EARLY IN LIFE?
1. The neonatal period is a critical early window for mother-to-baby transmission of gut microbial strains.

2. Caesarean-section delivery and antibiotics disrupt the vertical transmission of the maternal gut microbiota, including Bacteroides species. This may predispose colonization of opportunistic pathogens originating from the hospital environment; which can persist in the faecal microbiota (and therefore gut?) to infancy.

3. C-section babies are deficient in bifidobacteria and bacteroides-long term implications?

4. Breast-feeding has a greater effect on the microbiome of C-section babies

5. Potential for live biotherapeutics/polybiotic solutions to replace the missing microbes.

6. Microbiome of preterm babies is understudied and needs attention: Microbiome memory: 4 year olds still have an imprint of premature birth
Feeding the microbiome from birth

Human microbiome is inseparable from host health.

**Good start:** Natural birth, breast feeding by well-nourished mother and antibiotic avoidance.

**Maintenance:** diverse diet, limit junk food, adequate dietary fibre, exercise and moderation in all respects

**Source of Pharmabiotics** with potential to influence host health and shape the microbiota in ways antibiotics cannot-

Opportunities for interventions/diagnostics, for food, biotech & pharma
Acknowledgements


Drs. Kiera Murphy, Cian Hill, Denise Lynch, Ian Jeffrey, Claire Watkins, Amy Murphy, Grace Ahern, Katriona Lyons, Carol-Anne O’Shea, Aoife Collery, Caitriona Long-Smith, Grainne, Meehan, Finola Keohane, Kieran Tuohy, Marynka Ulaszewska,

Mother and Infant Cohorts