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“From Sciences to Nutrition Security”

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Human Microbiome, Sharing our diet: an Asian Perspective
(Disclosure: There is no conflict of interest)

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## Gut microbiota deviations associated with diseases or disease risk

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Microbiota at 0 -12 months</th>
<th>Microbiota at 24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autistic children</td>
<td>Higher numbers of <strong>clostridia</strong></td>
<td>Higher numbers of <strong>clostridia</strong></td>
</tr>
<tr>
<td>Wheezing infants</td>
<td>High <strong>clostridia</strong></td>
<td>Less diverse microbiota</td>
</tr>
<tr>
<td>Infants at risk of diarrhoeal</td>
<td>Low <strong>bifidobacteria</strong>, high <strong>clostridia</strong>, less diverse microbiota</td>
<td>Less diverse microbiota</td>
</tr>
<tr>
<td>Allergic infants</td>
<td>At 6 months lower <strong>bifidobacteria</strong> and higher <strong>clostridia</strong></td>
<td>Less <strong>lactobacilli</strong>, high numbers of <strong>aerobic bacteria</strong>, high <strong>coliforms</strong>, higher <strong>Staphylococcus aureus</strong> counts</td>
</tr>
<tr>
<td>Infants later developing allergic disease</td>
<td>Early microbiota (already at 2-3 weeks or 1 month) less <strong>bifidobacteria</strong>, and different species composition, often higher numbers of <strong>Bifidobacterium adolescentis</strong>, higher <strong>clostridia</strong></td>
<td>Differences similar but not so pronounced, but still present even at 5 years of age (including the higher numbers of <strong>B. adolescentis</strong> in allergic infants</td>
</tr>
<tr>
<td>Normal infants</td>
<td>At 6 months high <strong>bifidobacteria</strong>, low <strong>clostridia</strong> (especially in breastfed infants)</td>
<td>High numbers of <strong>aerobic bacteria</strong>, high diversity, number of unculturable bacteria increase</td>
</tr>
</tbody>
</table>
Diet provides nutrients to both human body as well as associated microbes, in particular gut microbiome.
### Asian Microbiome Project (AMP) Phase I initiated in 2009
Species abundance in 303 Asian children / 5 countries in Asia

**“Signature of country in gut microbiota” (MiSeq sequencing)**

<table>
<thead>
<tr>
<th>Log_{10}(relative abundance %)</th>
<th>Tokyo</th>
<th>Fukuoka</th>
<th>Taipe</th>
<th>Taichung</th>
<th>Beijing</th>
<th>Lanzhou</th>
<th>Bangkok</th>
<th>Khon Kaen</th>
<th>Yogyakarta</th>
<th>Bali</th>
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<tbody>
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</tr>
</tbody>
</table>

**Key Species**
- **Ruminococcus obeum**
- **Gemmiger formicilis**
- **Clostridium clostridioforme**
- **Blautia wexlerae**
- **Eubacterium hadrum**
- **Bacteroides vulgatus**
- **Bifidobacterium pseudocatenulatum**
- **Bifidobacterium longum**
- **Clostridium mayombei**
- **Bacteroides uniformis**
- **Bacteroides ovatus**
- **Eubacterium ventriosum**
- **Clostridium bartlettii**
- **Eggerthella lenta**
- **Phascolarctobacterium faecium**
- **Dorea formicigenerans**
- **Dorea longicatena**
- **Clostridium nexile**
- **Escherichia coli**
- **Coprococcus catus**
- **Butyrivibrio pullicaecorum**
- **Blautia stercoris**
- **Eubacterium biforme**
- **Prevotella copri**
- **Catenibacterium mitsuokai**
- **Desulfovibrio piger**
- **Sutterella stercoricanis**
- **Dialister succinatiphilus**
- **Methylobacterium populi**
- **Dialister invisus**

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**Note:**

- **I:** Rich in Ruminococcus obeum and Gemmiger formicilis, typical of Mongolian children.
- **II:** Rich in Eubacterium ventriosum and Clostridium bartlettii, typical of Chinese children.
- **III:** Rich in Eubacterium biforme and Prevotella copri, typical of Thai children.
- **IV:** Rich in Methylobacterium populi and Dialister invisus, typical of Indonesian children.
PCA and clustering of fecal microbiota of 303 Asian children, 7-11 yr (at family level)

Prediction strength = 0.90
Silhouette = 0.313

BB-enterotype
P-enterotype

relative abundance (%)

Beijing
Lanzhou
Tokyo
Fukuoka
Taipei
Taichung
Bangkok
Khon Kaen
Yogyakarta
Bali
Gut microbiome of Asian children across geographical region

Random forest clustering of 303 Asian children

Dimension 1

Dimension 2

Percentage = accuracy in random forest clustering

Enterotypes:

Type 1: Consumed lots of meat & saturated fat - more Bacteroides

Type 2: People who consumed lots of alcohol & polyunsaturated fats - Ruminicoccus prevailed

Type 3: Diet rich in carbohydrates - favored Prevotella

Linking long-term dietary patterns with gut microbial enterotypes
Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

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(C)

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(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)

Impact of modern high-meat/-fat diet on gut microbiota in children on Leyte island Philippines

(A)

(B)

(C)
Redundancy analysis to correlate macronutrient intake with gut microbiota

Enrichment of genes involved in bile acid metabolism in BB-enterotype

Choloylglycinehydrolase (CGH)

Bile acid-coenzyme A ligase (BaiA)
**Enterotypes:**

**Type 1:** Consumed lots of meat & saturated fat - more Bacteroides?? Mongolian

*A secondary dietary factor?*
<table>
<thead>
<tr>
<th>Staple carbohydrate</th>
<th>Insoluble fibre (g/100 g)</th>
<th>Amylose (% total starch)</th>
<th>Starch fraction (% dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amylose</td>
<td>Rapidly digestible</td>
</tr>
<tr>
<td>Mongolia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>12.0</td>
<td>29</td>
<td>24.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>14.7</td>
<td>26</td>
<td>38.1</td>
</tr>
<tr>
<td>Oat</td>
<td>33.9</td>
<td>26</td>
<td>35.8</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>7.0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Millets</td>
<td>3.1</td>
<td>21</td>
<td>35.9</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td>3.1</td>
<td>21</td>
<td>35.9</td>
</tr>
<tr>
<td>Sorghum</td>
<td>4.2</td>
<td>24</td>
<td>29.2</td>
</tr>
<tr>
<td>Black-eyed peas</td>
<td>32.4</td>
<td>38</td>
<td>18.5</td>
</tr>
<tr>
<td>Indonesia Thailand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indica rice</td>
<td>1.2</td>
<td>33</td>
<td>32.0</td>
</tr>
<tr>
<td>Japan Korea China</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japonica rice</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat flour</td>
<td>8.5</td>
<td>26</td>
<td>38.1</td>
</tr>
<tr>
<td>Italy</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Wheat flour</td>
<td>8.5</td>
<td>26</td>
<td>38.1</td>
</tr>
<tr>
<td>USA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>1.1</td>
<td>20</td>
<td>75.5</td>
</tr>
</tbody>
</table>

**Resistant starch**: RS1- starch in seeds or legumes and unprocessed whole grains; RS2- natural granular form, e.g. high amylose corn; RS3- retrograded cooked starch. Drives Prevotella!

Food intake (Carbohydrate) frequency (per day)

Japonica

Resistant Starch (0.6%)

Rice

<table>
<thead>
<tr>
<th>City</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing</td>
<td>1.4</td>
</tr>
<tr>
<td>Lanzhou</td>
<td>1</td>
</tr>
<tr>
<td>Tokyo</td>
<td>1.9</td>
</tr>
<tr>
<td>Fukuoka</td>
<td>2.1</td>
</tr>
<tr>
<td>Bangkok</td>
<td>2.4</td>
</tr>
<tr>
<td>Khon Kaen</td>
<td>2.4</td>
</tr>
<tr>
<td>Yogyakarta</td>
<td>2.9</td>
</tr>
<tr>
<td>Bali</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Wheat

<table>
<thead>
<tr>
<th>City</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing</td>
<td>1.1</td>
</tr>
<tr>
<td>Lanzhou</td>
<td>1.2</td>
</tr>
<tr>
<td>Tokyo</td>
<td>0.9</td>
</tr>
<tr>
<td>Fukuoka</td>
<td>1</td>
</tr>
<tr>
<td>Bangkok</td>
<td>0.6</td>
</tr>
<tr>
<td>Khon Kaen</td>
<td>1.1</td>
</tr>
<tr>
<td>Yogyakarta</td>
<td>0.8</td>
</tr>
<tr>
<td>Bali</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Indica

Javanica

Resistant Starch (6.6%)

>: higher than other cities ($p<0.05$); >>: higher than other cities ($p<0.001$)

<: lower than other cities ($p<0.05$); <<: lower than other cities ($p<0.001$)
Predicted metagenome (PICRUSt) suggests non-digested amylose in the colon of P-type

*K01176 (alpha-amylase)

*K07405 (alpha-amylase)

*K01187 (alpha-glucosidase)

*Pie chart represents contribution of bacteria family to these genes
WORKING HYPOTHESIS

Relative proportion of dietary fat & resistant carbohydrate

**BB-type**
Less resistant starch $\Rightarrow$ ↑ Bile acid in colon $\Rightarrow$ Kill *Prevotella* + bile sensitive species $\Rightarrow$ ↓ Diversity

**P-type**
High resistant starch $\Rightarrow$ ↓ Bile acid in colon $\Rightarrow$ Promote *Prevotella* + bile sensitive species $\Rightarrow$ ↑ Diversity
Microbial heritage: enhanced by Mother to infant transfer?


Yatsunenko et al., Nature 2012

中国四川地区儿童肠道菌群. Heping Zhang

Yatsunenko et al., Nature 2012
Take Home Message

• Diet determines enterotype.
• Which is the healthier enterotype, 
  
• *Prevotella* enterotype caries GI pathogens !?
**Correlation between gut commensal and pathogens**

- **Red line** indicate negative correlation
- **Blue lines** indicate positive correlation

**Diet induced cancers & circular-vascular diseases** occurs more frequently in Europe and North America.

**Clostridium difficile colitis**

**IBD**

**Pneumonia, necrotizing fasciitis**

**Crohn’s disease**

**Graft vs host disease**

**Appendicitis, IBD**

Infectious diarrheal and necrotizing enterocolitis, occurs more frequently in developing Africa and Asia, among children and preterm infants.
Relative abundance of potentially pathogenic bacteria in the 10 cities

"East Asian youngsters harbor less amount of potentially pathogenic bacteria"

Hygiene?

![Box plot showing relative abundance of potentially pathogenic bacteria across 10 cities.](chart.png)
Gut microbiota of healthy population worldwide

**Bacteroidaceae**
- Fukuoka
- Tokyo
- Korea
- Taipei
- Taichung
- Beijing
- Lanzhou
- Bangkok
- Khon Kaen
- Bali
- Mongolia
- Sichuan
- Burkina Faso
- Florence
- USA
- Bangladesh

**Bifidobacteriaceae**

**Ruminococcaceae**

**Lachnospiraceae**

**Prevotellaceae**

Diet determines enterotype

**Bacteroides** the healthier enterotype?

Commensal strengthen epithelial layer tight-junction (increase expression of zonula occludens-1 & myosin light-chain kinase), preventing crossover of pathogen and LPS, chronic inflammation and type-2 diabetes.
WORKING HYPOTHESIS

Relative proportion of dietary fat & resistant carbohydrate determine enterotype

P-type
High resistant starch $\Rightarrow$ ↓ Bile acid in colon $\Rightarrow$ Promote *Prevotella* + bile sensitive species $\Rightarrow$ ↑Diversity

BB-type
Less resistant starch $\Rightarrow$ ↑ Bile acid in colon $\Rightarrow$ Kill *Prevotella* + bile sensitive species $\Rightarrow$ ↓Diversity

Over consumption of fat led to obesity

Pathogen-enriched
High fat $\Rightarrow$ ↑ Bile acid/ fatty acids in colon $\Rightarrow$ Kill commensal, weaken tight junction $\Rightarrow$ ↑Chronic inflammation
Does obesity play any role in Type-2 Diabetes in Asia? Over consumption of carbohydrate could lead to obesity! Starch interfere fat digestion in the gut.
WORKING HYPOTHESIS

Relative proportion of dietary fat & resistant carbohydrate determine enterotype

**P-type**
- High resistant starch $\rightarrow$ Bile acid in colon $\rightarrow$ Promote *Prevotella* + bile sensitive species $\rightarrow$ Diversity

**BB-type**
- Less resistant starch $\rightarrow$ Bile acid in colon $\rightarrow$ Kill *Prevotella* + bile sensitive species $\rightarrow$ Diversity

**Over consumption of fat led to obesity**

**Pathogen-enriched**
- High fat $\rightarrow$ Bile acid/ fatty acids in colon $\rightarrow$ Kill commensal, weaken tight junction $\rightarrow$ Chronic inflammation

**Over consumption of carbohydrate led to obesity**
- High carbohydrate $\rightarrow$ Bile acid/ fatty acids $\rightarrow$ Commensal remained, strengthen tight junction $\rightarrow$ No chronic inflammation
Implication?

High fat-low resistant starch (wheat, potato) consumers eat more resistant starch (Indica rice, bailey, oat, millet) to prevent type-2 diabetes.

High resistant starch-low fat consumers switch to low resistant starch or eat more fat to prevent MCI/dementia and improve on longevity. (hypertension, cardiovascular diseases?!) (Working hypothesis for intervention study!)
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