The Gut Microbiota and Host Metabolic Physiology / Growth

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Nutrition: a quintessential sustainable development goal

34 countries harbor 90% of the child malnutrition burden

3.1 million annual deaths under the age of five (~45% of under-five mortality)

Defining nutritional status according to the World Health Organization


WHO Multi-Center Growth Reference Study from approximately 8500 children from widely differing ethnic backgrounds and cultural settings (Brazil, Ghana, India, Norway, Oman and the USA) assessing the growth and development of infants and young children around the world.

Z-scores:
Height-for-Age
Weight-for-Age
Weight-for-Height

A single international standard that is the best description of growth for all children from birth to five years of age.
Defining nutritional status according to the World Health Organization

Diagnostic criterion and presentation of Severe Acute Malnutrition:

- WLZ score < -3
- Bipedal edema
- MUAC < 11.5 cm

Therapeutic foods to treat malnutrition

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<th></th>
<th>RUTF</th>
<th>Khichuri</th>
<th>Halwa</th>
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<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>530-545</td>
<td>145</td>
<td>240</td>
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The gut microbiota as part of the vicious cycle of malnutrition

Undernutrition (malnutrition) is not caused by food insecurity alone, but by a variety of intra- and inter-generational factors, all of which could be influenced by the gut microbiota.
The gut microbiota can be thought of as another organ in the human body.

The human gut microbiota: community of microbes that live along the human gut.

Microbiome refers to the genetic sequence of these microbes.

**Food** shapes the balance of power in this community and can in turn affect:
- Nutritional absorption
- Metabolism
- Immunity and Vaccination
- Susceptibility to Infections

**Central Hypothesis:**
Human gut microbes develop like the rest of our organs, and this development is affected in undernutrition.
Microbes in turn influence host physiology and development

Villi microvascular network regulated by microbes via Paneth cells

- Gut barrier function
- Modulation of regulatory T cells and other immune cell populations
- Intestinal motility
- SCFA production
- TMAO production, tryptophan, bile salt metabolism

Stappenbeck, Hooper and Grodon, PNAS 2002
Gut microbial communities change dramatically in early life

Age is a major factor in shaping what bacterial communities look like in the gut

Based on a pair-wise phylogenetic distance based approach, completely **unsupervised**, children approximate their parents over the time course of two years

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Yatsunenko et al., 2012
Collaboration to study undernutrition in Mirpur, Bangladesh

Collaboration with ICDDR,B

Oral Rehydration therapy was discovered here, now implemented worldwide as life-saving therapy for cholera.

Study participants are inhabitants of an urban slum area in Mirpur, Dhaka

Birth cohort and nutritional status data were collected along with biospecimens.

Photos reproduced with consent courtesy of icddr,b Bangladesh
Methods to characterize the bacterial component of the gut microbiota

Pulverize bio-specimens & extract genomic DNA

Biospecimens provide a non-invasive measure of bacterial diversity in the gastrointestinal tract

PCR amplify sample DNA using a 16S rRNA gene (phylogenetic ‘barcode of bacterial life’)

Group related sequences into Operational Taxonomic Units (OTU) based on alignment with 97% sequence identity.

Bacterial species/strains/taxa are defined based on 97% identity
Question: Given a healthy child’s diaper biospecimen, can we accurately predict the age of a child? Can we use this as a measurement of a child’s gut development?

Random Forests: decision-tree based machine learning method applied to identify bacterial strains that can serve as markers of the aging process.

Advantages:
1. Non-parametric assumptions
2. Ability to deal with large number of features relative to data-points (predictors >> number of data points)
   1. Provides a ranking of features that assist with feature selection
   2. Suited for sparse dataset types (tables with lots of 0’s)

Disadvantages:
black-box nature of algorithm, possibility of over-fitting, limited by the data provided for training
Defining the stages of gut microbiota development using RF regression

Healthy infants/children from Bangladeshi study population; sampled monthly from birth to two years

Use Random Forests (machine learning) to identify bacterial taxa that are most discriminatory for different stages in assembly of the microbiota

Create sparse model based on most age-discriminatory taxa

Each row is a different age-discriminatory strain

Y is age of child ~ Xi are the abundances of each type of gut bacteria, i
Identification of bacterial taxa as biomarkers of healthy gut microbiota maturation

Steps to identification of age-discriminatory taxa:

1. **Rank bacterial taxa** in order of mean-squared error, permutation-based importance score

2. **Cross-validate** within training set to estimate number of taxa needed for accurate prediction

3. **Generate** sparse model and **validate** model in samples from a different set of unrelated children
Validation of a sparse Bangladeshi model consisting of age-discriminatory taxa

Starting materials:

Samples from healthy children (25 singletons, 11 twin pairs, 1 set of triplets; 996 fecal samples; 50 children)

Subramanian et al., 2014
Definition of two microbiota metrics based on the Bangladeshi model

(1) Relative microbiota maturity = microbiota age of a given child – microbiota age for healthy children of similar chronological age

(2) Microbiota-for-age Z score (MAZ) = (predicted microbiota age – median) / standard deviation of microbiota age
Application of metrics to a cohort of Bangladeshi twins and triplets

Diarrheal episodes in anthropometrically healthy children are associated with a transient reduction in maturity levels that recovers.

ANOVA of linear mixed models: ‘diarrhoea’, ‘month following diarrhoea’ and ‘presence of formula in diet’ have significant effects, *P < 0.05, **P < 0.01
Presentation of severe acute malnutrition (SAM)

- Weight-for-Length Z score < -3 SD
- Bipedal edema, MUAC < 11.5 cm

Stabilization with Milk Suji (porridge)

Oral Rehydration

Antibiotics

Administration of therapeutic foods

Plumpy-Nut

RUTF

Khichuri

Halwa
Inpatient treatment of children with severe undernutrition
Randomized clinical trial comparing two food interventions to treat SAM

Despite treatment, children don’t improve in terms of age-adjusted height and not completely with weight.
Persistent immaturity of the gut microbiota in children with SAM

Acute Phase of SAM

- n = 31 children
- n = 22 of 31 children

RUTF

Post-intervention follow-up

Khichuri-Halwa

Post-intervention follow-up

b

Healthy children (> 6 mo)

At enrollment

End of RUTF intervention

End of Khichuri-Halwa intervention

Months of follow-up

< 1 1-2 2-3 3-4 >4

At enrollment

Khichuri-Halwa

Months of follow-up

< 1 1-2 2-3 3-4 >4

Relative microbiota maturity (mo)
Microbiota immaturity evident prior to administration of antibiotics
Conclusions from Bangladesh study

Two types of therapeutic foods produce an improvement in microbiota maturity indices that was not sustained.

More prolonged food-based interventions of varying composition and/or addition of gut microbes may be beneficial.

Age-discriminatory taxa may themselves serve as therapeutic agents and/or targets of next generation microbiota-directed therapeutic foods.
Gut microbial milestones as metrics to monitor therapeutic interventions

Bacterial strains discriminatory for age in healthy children provide a way to characterize malnourished states and human postnatal development.

Beyond their diagnostic value these features can be now investigated in terms of their functional roles in cell-based, animal and human studies.

Subramanian et al., 2015
Future questions?

Relationship between maternal microbes and infant microbes?

Relationship between complex food consumption patterns and gut bacteria?

Relationship between gut microbial organ development and development of other organs? Brain, Bone, Immune System Development

Subramanian et al., 2015
Testing causality in a humanized gnotobiotic model of kwashiorkor

Malawian twin pair discordant for kwashiorkor

Smith, Yatsunenko et al., Science 2013
Testing causality in a humanized gnotobiotic model of stunting

Malawian singletons with chronic forms of undernutrition

Blanton et al., Science 2016
Pleiotropic effects in humanized mouse models of undernutrition

Charbonneau et al., Cell 2016
Neonatal sepsis and an early synbiotic interventions

A randomized synbiotic trial to prevent sepsis among infants in rural India

Community-based, double-blind, placebo-controlled randomized trial in 149 randomly chosen villages in Odisha state
Microbiota directed complementary foods for undernutrition
Preserving and nurturing our resources for a sustainable future