

Introduction

Establishment and succession of the human microbiome begins at birth and microbial composition adapts alongside human development and growth. Development of a healthy signature microbial community has significant effects on health. This study followed 18 very low birth weight (VLBW) infants, measured initially in the Neonatal Intensive Care Unit (NICU), at the age of two to three years old.

Objective

This study explored correlations among the toddler gut microbiome of formerly very low birth weight infants and health status variables.

Methods

- Approval obtained from the university IRB and informed consent was obtained from the mothers.
- We followed VLBW infants (<1500 gms) during 6 weeks of the NICU stay and followed up with home visits and stool collection at 2-3 years of age on 18 toddlers.
- We analyzed relationships between the toddler gut microbiome and health variables (hemoglobin, frequency of illness during the first year of life, and introduction of solid foods) collected when they reached 2-3 years of age.
- Eighteen stool samples from these children when they reached 2-3 years of age (mean age =31 months old) have been sequenced.
- Sample process: Stool swab samples were stored at -80°C prior to use. Microbial genomic DNA was extracted using the PowerSoil-htp 96 Well Soil DNA Isolation Kit (MoBio). DNA Samples were forwarded to the lab of Dr. Jack Gilbert (Argonne National laboratory and University of Chicago). The microbial content was analyzed with 16SrRNA sequencing. PCR of the 16S rRNA V4 region (515F-806R) was performed and sequenced using the Illumina MiSeq platform to generate ~100,000 250 bp paired end reads per sample.

Results

- 18 Toddlers formerly VLBW infants (<1500 Gms) with equal gender distribution.

Table 1. Study population Characteristics

Characteristics	Mean	Range
Gestational age (weeks)	28.3	25.3-32.4
Birth weight (gms)	1115.06	675-1485
Length of stay (days)	68.67	22-123
Days on Oxygen (days)	14.3	0-101

- Phyla composition: 54.12% *Firmicutes*, 32.74% *Bacteroidetes*, 5.41% *Actinobacteria*, 3.61% *Proteobacteria*, and 3.48% *Verrucomicrobiota*.
- Measures of Firmicutes strongly predicted 74% of the variance in Bacteroidetes.

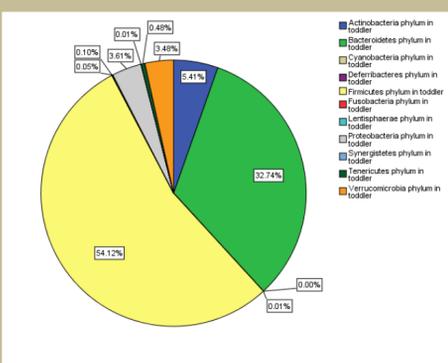
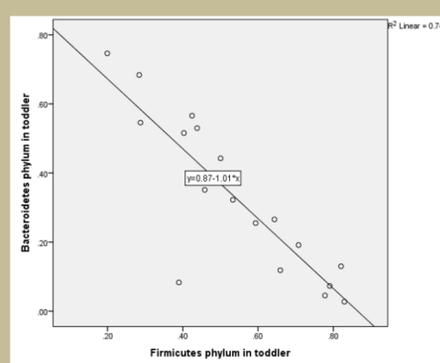


Figure 1. Bacterial taxonomy in toddler



Graph1. Firmicutes and Bacteroidetes relationship

Results

- The toddler hemoglobin level was related to Bacteroidetes abundance ($r=.479$, $p=.044$, $R^2=0.229$) and inversely related to Firmicutes ($r=-.516$, $p=.028$, $R^2=0.266$) and Actinobacteria ($r=-.697$, $p=.001$, $R^2=0.486$) abundance in toddlers.
- Remaining OTU abundances showed weak or non-significant correlations to Hgb.

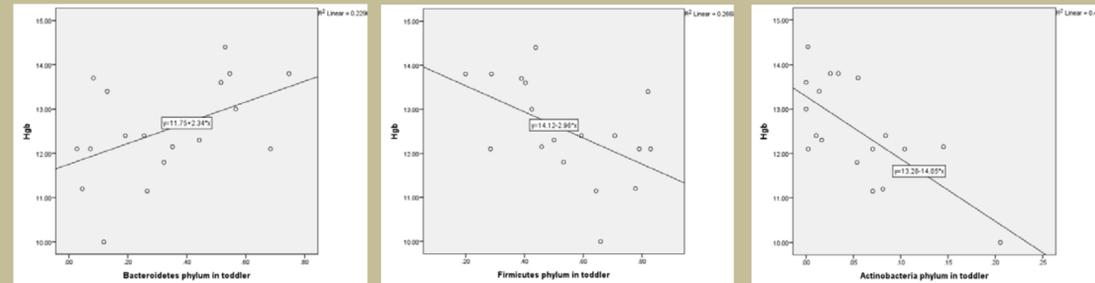


Figure 2. Relationship between Hemoglobin Level and OTU abundances

- Frequency of febrile illness was related to Actinobacteria abundance and age of introduction to solid foods was related to Tenericutes abundance.

Table 1. Correlations of Health Variables and Gut microbiome

		Age in months started eating solid food	Frequency of fever in the first year of life
Actinobacteria phylum in toddler	Pearson Correlation		0.609
	Sig. (2-tailed)		0.007
Tenericutes phylum in toddler	Pearson Correlation	0.804	
	Sig. (2-tailed)	0.000	

a. N=18.

- We used multiple regression to assess the unique roles played by frequency of fever in the first year of life, age, and hemoglobin level on Actinobacteria abundance. By eliminating the effect of other independent variables, the frequency of febrile illness in the first year of life and hemoglobin level showed moderate correlation to Actinobacteria abundance in toddler.

Table 2. Regression analysis related to Actinobacteria abundance in toddler

	Unstandardized Coefficients		Standardized Coefficients	F	Sig.	Correlation r sp
	B	Std. Error	Beta			
(Constant)	0.382	0.131		8.519	0.011	
Age_child	-0.001	0.002	-0.059	0.147	0.707	-0.059
Hgb Hgb	-0.028	0.008	-0.572	12.690	0.003	<u>-0.547</u>
Frequency of fever during the first year of life	0.026	0.009	0.446	7.723	0.015	<u>0.426</u>

a. Dependent Variable: Actinobacteria phylum in toddler

Discussion

- Firmicutes, Bacteroidetes, and Actinobacteria constitute the most abundant phyla in the human intestinal microbiota.
- The correlation between hemoglobin level and the gut microbiome suggests that certain species of gut bacteria could influence iron absorption and hematopoiesis. It is also possible that diet and illnesses play roles in this relationship. We are currently examining these relationships.

Conclusion

- The gut microbiome is an important regulator of physiological and pathophysiological effects. Further research is necessary regarding children's health and hemoglobin relationships with gut microbiome.

References

- Zijlmans, C. W. R., Stuursma, A., Roelofs, A. J., Jubitana, B. C., & MacDonald-Ottevanger, M. S. (2017). Anemia in young children living in the Surinamese interior: the influence of age, nutritional status and ethnicity.
- Hidalgo-Cantabrana, C., Delgado, S., Ruiz, L., Ruas-Madiedo, P., Sanchez, B., & Margolles, A. (2017). Bifidobacteria and Their Health-Promoting Effects. *Microbiology spectrum*, 5(3)

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