Association between preterm infant gut microbiome and growth failure

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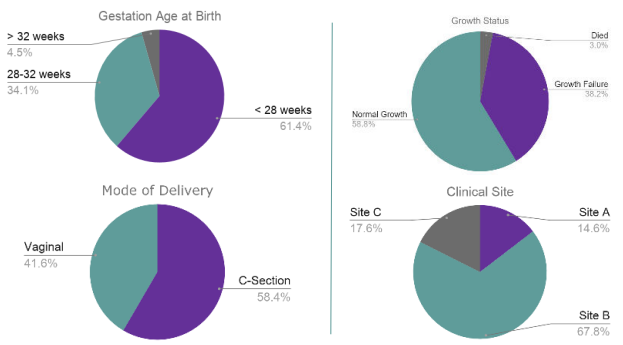
Background

- Preterm infants in the NICU present unique challenges for clinical management of growth due to their increased antibiotic exposure, requirement for parenteral nutrition, and need for mother's own milk, which is often insufficient.
- These factors are associated with growth failure (GF), defined as a birth-to-discharge weight z-score decline of ≥1.2.
- The developing gut microbiome is thus perturbed in preterm infants, which may lead to adverse outcomes.
- We hypothesized an association between the preterm gut microbiome and infant growth rate in the NICU (1).

Study Design

- **Study Population**: 267 preterm infants from 3 different clinical sites with associated data from birth to hospital discharge.
- **Sample collection**: Stool samples (n=2947) were collected longitudinally from 1 to 174 days of life, from infants with normal growth (GN, n= 157), GF (n=102) and infants who died (n=8). See background for GF definition.
- **Sample Processing**: Extracted DNA was sequenced via shotgun metagenomic sequencing at a mean depth of 28,390,685 sequences.
- **Data Generation:** Shotgun sequencing was annotated using MetaPhlAn2 and HUMAnN2 (2, 3).
- **Covariates:** Growth Status, Clinical Sites, Probiotic (yes/no), Sepsis, Necrotizing Enterocolitis, Mode of Birth, Gender, Gestational Age at Birth, Post Menstrual Age.

Data Set



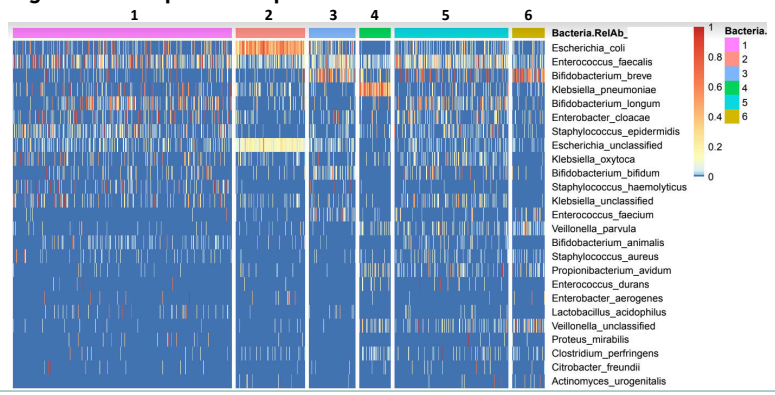
Percentages are derived from infant-level data.

Results

Dirichlet Multinomial Mixtures Identified Six Preterm Gut Community Types

- Dirichlet multinomial mixtures (DMM) was applied to cluster samples and identified six distinct microbiome "preterm gut community types" (PGCTs) based on the overall bacterial profiles (4).
- PGCTs are numbered 1-6 according to the average age of samples within each PGCT, with PGCT-1 consisting of the earliest samples.

Figure 1. Heatmap of Six Unique Clusters



Based on the identified clusters Fig. 1 shows a heatmap highlighting the relative abundance of various species within each of the six clusters. PGCT-1 contains the least number of species and has the lowest Shannon diversity.

Some of the PGCTs are dominated by a single taxa, for ex. *Escherichia_coli* for PGCT-2 has 50% relative abundance, *Klebsiella_pneumoniae* in PGCT-4 has relative abundance of 53%.

PGCT	Mean DOL	Mean Num Species
1	25.9	12.3
2	36.6	17.9
3	38.1	18.4
4	42.8	18.6
5	45.4	22.1
6	52.1	28.7

Figure 2. Transitions Among PGCTs Over Time

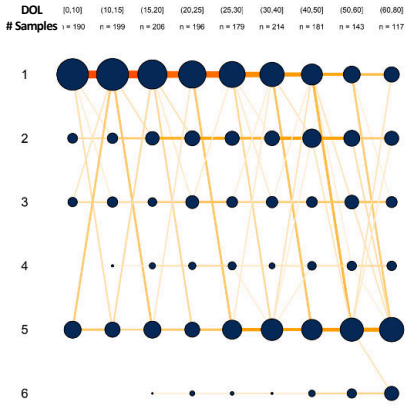


Fig. 2 highlights transitions between PGCTs over time. To avoid repeated measures, discrete time bins were chosen based on day of life (DOL) 0-9, 10-14, 15-19, 20-24, 25-30, with one sample per infant chosen within each time bin.

The density of the circles is representative of the number of samples in the PGCT in that particular time bin. Line color and density are representative of transition frequency.

Most infant samples begin in PGCT-1 and frequently remain there until they transition out, often into PGCT-5.

Association Between PGCTs and Growth Failure

- We separated samples into time bins – grouped by PGCTs, PGCT-1 vs the others, and used percent growth normal to calculate the relative risk (RR) of being GF vs GN in the different time windows.
- Multiple testing correction of P-values with Benjamini-Hochberg (BH) FDR.
- We find that infants in PGCT-1 are significantly more likely to have growth failure, with ~ 1.64 Relative Risk Ratio after the first 30 DOL.

Time Period	PCGT Bins	Average %GN 1 vs [2-6]	RR 1 vs [2,6] (95% CI)	RR P-value (BH –adj)
DOL [30,40]	1	44%	**1.64** (1.2-2.2)	**0.008**
	[2-6]	66%		
DOL [40,50]	1	40%	**1.64** (1.2-2.2)	**0.008**
	[2-6]	63%		

Next Steps/ Future Work

- Further exploration of the association between PGCTs and GF using robust statistical techniques to incorporate all samples.
- Further investigation of PGCT-1, as it has a strong association with growth failure.

Conclusions

- Preliminary results demonstrate the potential to define distinct gut bacterial community types in preterm infants, which may have value for predicting growth status and informing clinical care to enhance growth.
- Further work is needed to validate these findings and explore if modulating the gut microbiome in the first 30 days of life can improve growth outcomes in preterm infants.
- The bacterial species that were enriched in PGCT-1 include several pathobionts (without proof/claim of 'causality') for ex: *Enterococcus_faecalis, Escherichia_coli, Staphylococcus_epidermidis, and others.*

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