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Kangaroo Mother Care and the Brain-Gut-Microbiota Signaling System

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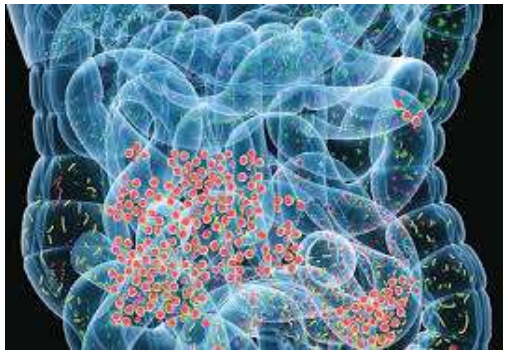
Objectives:

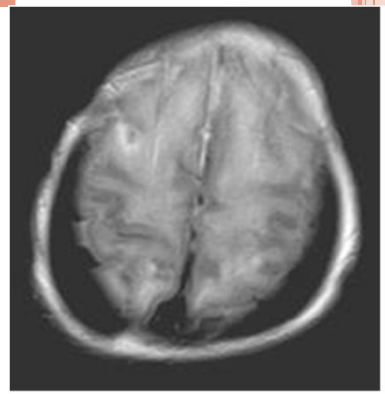
1. Explain “omic” conceptual framework for studying early life stress.

2. Describe innovative designs for including omic measures in KMC research.

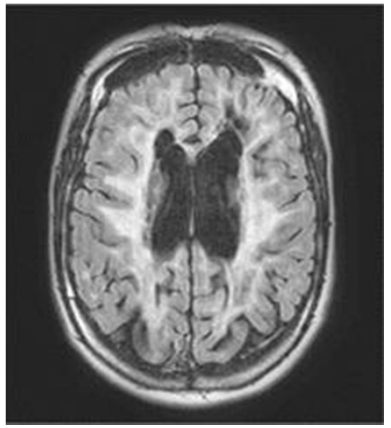
3. Identify translational opportunities for applying research findings to reduce neonatal stress and improve KMC practice.



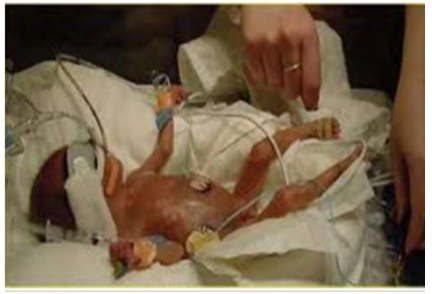
Early Life Experience in the NICU



- The U.S. ranks one of the highest in the world for the number of preterm births.
  < 37 weeks of gestation; 10% in 2014



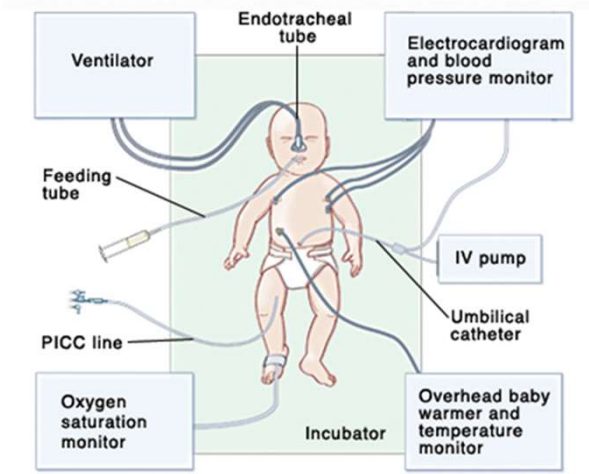
- In NICU, infants are exposed to numerous early life stressors during critical periods of neurodevelopment.
  Maternal-Infant separation
- 40% NICU survivors have at least 1 neurodevelopmental deficit.

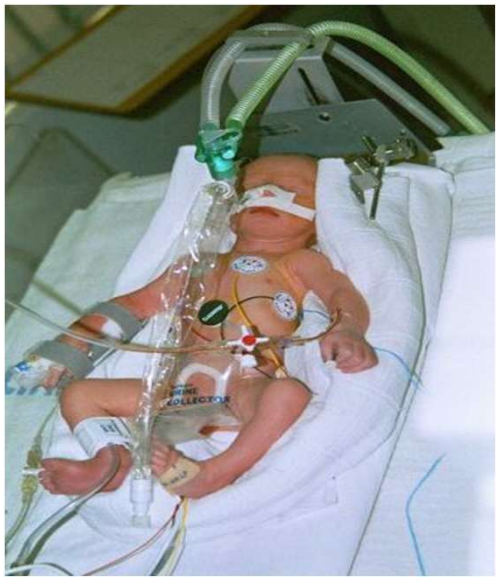


- **Yet, mechanisms of early life experiences that alter infants' health outcomes remain largely unknown.**

Stressors in the NICU

- Separation from parents and family
- Repeated invasive painful procedures
- Daily uncomfortable cares
- NICU environment - noise, light, infection





Early Life Physiological and Psychosocial Stress Imprints Gut Microbiome in Preterm Infants

To investigate the regulation of early life stress by the brain-gut-microbiota signaling mechanism and explore non-invasive microbial and immune-inflammatory predictors of neurodevelopment.

Gut Microbiome

- Gut microbiota influences many aspects of human health:
  - Promote mucosal barrier protection,
  - Growth of blood vessels,
  - Energy harvesting and storage,
  - Defense against pathogens,
  - Food metabolism,
  - Regulation of blood pressure,
  - Innate immunity
- The underlying mechanism that affects gut microbiome patterns among preterm infants largely remains unknown.
- Gut Microbiome: the microbiota and the habitat it colonizes; the collective genomes of the microbes or the metagenome.

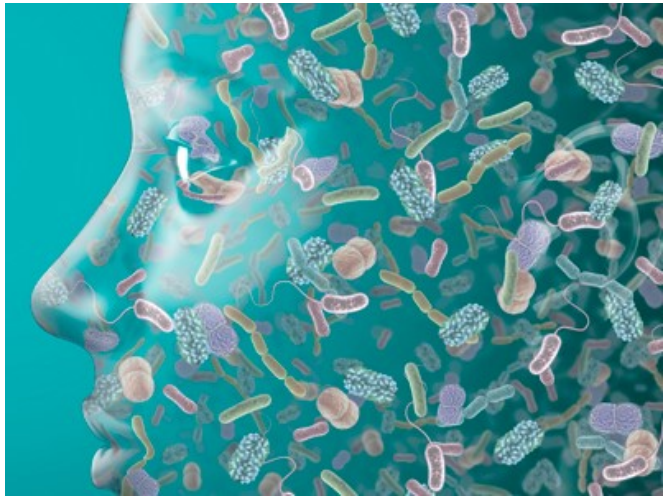
The Brain-Gut-Microbiota Axis

- **Components:** central nervous system, hypothalamus-pituitary-adrenal axis, sympathetic-parasympathetic autonomic nervous system, enteric nervous system, and intestinal microbiota.

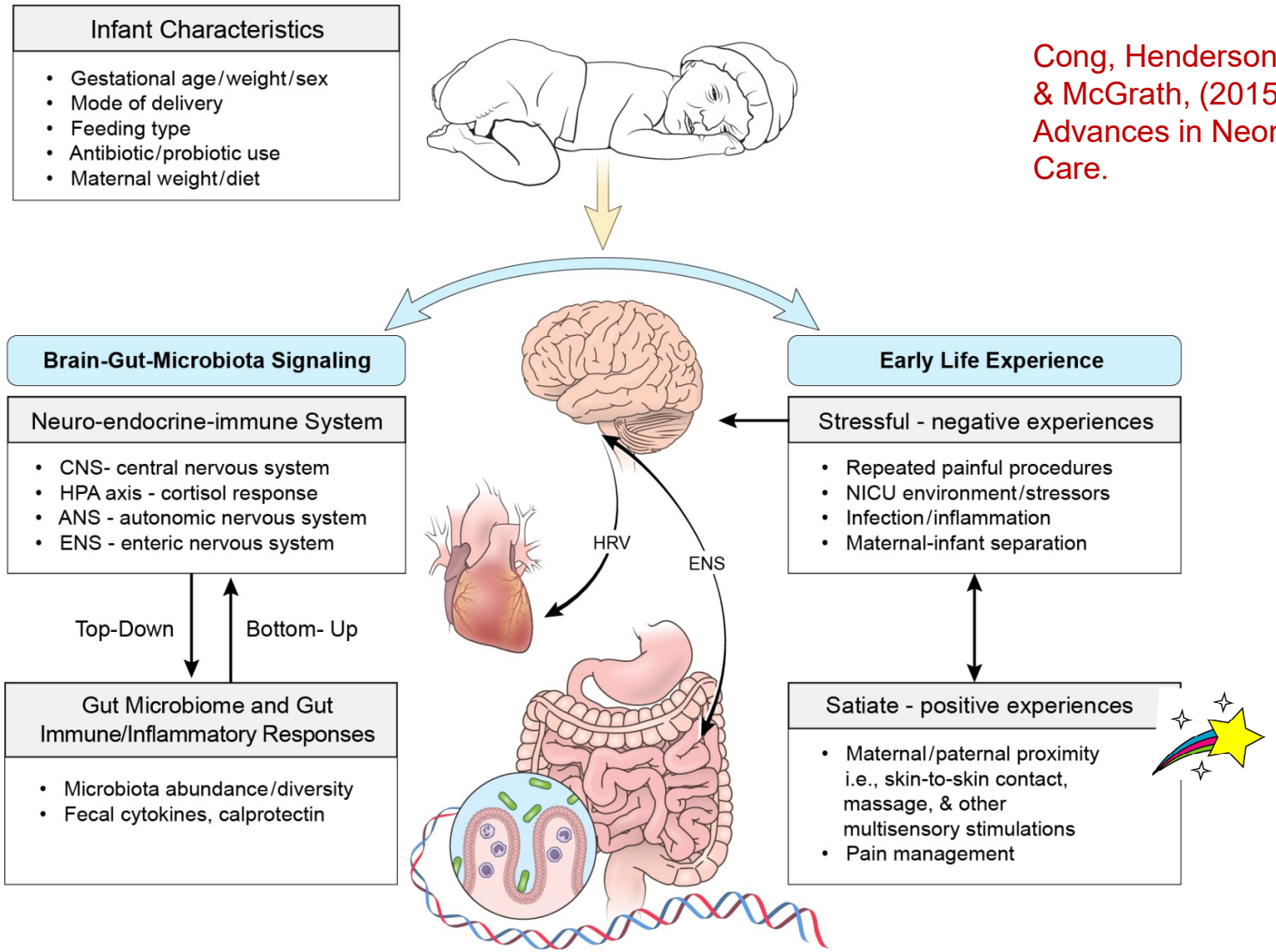
- Bidirectional communication network:

  Top-down: brain to influence the motor, sensory and secretory modalities of the GI tract.

  Bottom-up: gut to affect brain function (hypothalamus and amygdala).



Brain-Gut-Microbiota Signaling System



Cong, Henderson, Graf, & McGrath, (2015), Advances in Neonatal Care.

Gut Microbiome Patterns in Infants

- Colonization begins with facultative anaerobic organisms, followed by the development of obligate anaerobes, including *Bifidobacterium*, *Bacteroides*, and *Clostridium*.
- Full-term, breast-fed infant serves as the health standard or the "norm" for newborns.
- ***Factors:*** delivery mode, feeding, medication use, hospital environment, other early life experiences, and host genetics.

Gut Microbiome Patterns in Infants

- *Dysbiosis* of gut microbiota persists during infancy, especially in preterm infants, and then may reach a stable configuration at age 2 - 3 yrs.
- Preterm infants: demonstrate reduced levels of obligate anaerobes.
- Preterm infants: increased levels of facultative anaerobes, i.e., *Enterobacteriaceae* and *Enterococcaceae*
- https://www.youtube.com/watch?v=Pb272zsixSQ

Literatures

There is very limited research related to the effects of direct contact (i.e., KMC and direct breastfeeding) on the development of the infant microbiome development.

Author /Year	Country	Factors	Participants	Measures	Outcomes
Hendricks-Munoz, K.D., et. al., (2015).	United States	Skin to skin contact	42 preterm infants (<32 weeks of gestation at birth)	Saliva swabs were collected at 1 month of life and/or at discharge	KMC led to an increased pace of oral microbe repertoire maturity development and lower prevalence of organisms associated with intestinal dysfunction

Hendricks-Munoz, K.D., et al., Skin-to-Skin Care and the Development of the Preterm Infant Oral Microbiome. Am J Perinatol, 2015. Nov; 32(13):1205-16.

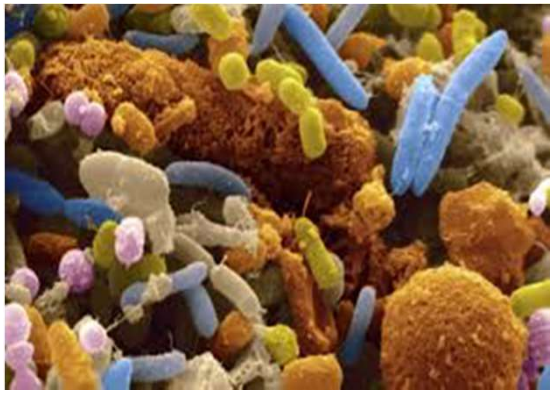
Cong, X., et al., Gut Microbiome and Infant Health: Brain-Gut-Microbiota Axis and Host Genetic Factors. Yale J Biol Med. 2016 Sep 30;89(3):299-308. eCollection 2016.

Study Aims

Aim 1: Determine preterm infants' gut microbiome patterns over first 3-4 weeks

Aim 2: The linkage of gut microbiome patterns with early life stress (i.e., KMC, pain, feeding)

Aim 3: The linkage of gut microbiome with neurodevelopmental outcomes.



Methods

- Design: Prospective longitudinal study.
- Setting: Level IV CCMC NICU at two sites, Hartford and Farmington, CT.
- Subjects: Stable preterm infants (26 – 32 weeks gestation), follow-up for 3-4 weeks.

Methods

- IRB approval and obtain consent from parents.
- Early life experience: KMC are measured daily

  NISS: Neonatal Infant Stressor Scale (Newnham, et. al, 2009), modified by our research team.
- Feeding types (Mother's, Donor's, Formula)
- Neurodevelopmental outcomes, at 36-38 weeks CA.

  NNNS: NICU Neurobehavioral Scale (Lester, et. al, 2004)

Methods

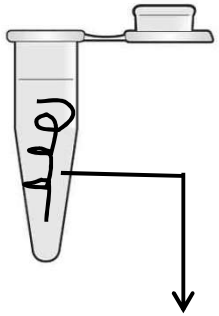
- Stool samples are collected daily starting 0 – 5 postnatal days for 3-4 weeks.

  Culture-independent DNA-based Genomic Technologies:

  Gut microbiota community profiles are determined by 16S rRNA sequencing and analysis

Investigating the Infant Microbiome

http://www.reuters.com

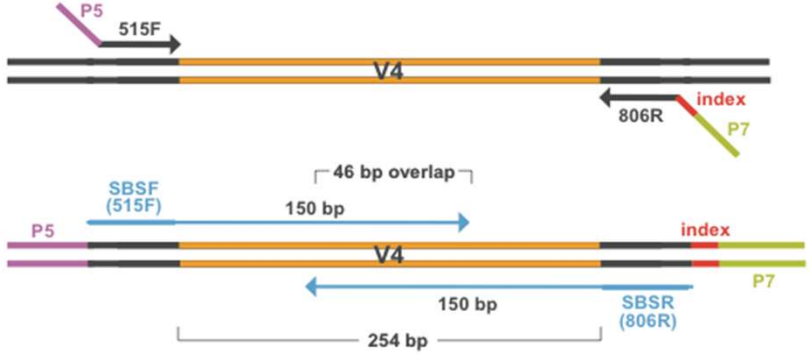


DNA Isolation from Microbial Community within Fecal Sample

PCR Amplification of the V4 Hypervariable Region of the 16S rRNA Gene

Illumina MiSeq Amplicon Sequencing

Illumina.com



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Quantitative Insights Into Microbial Ecology

qiime

Microbial Composition Analyses

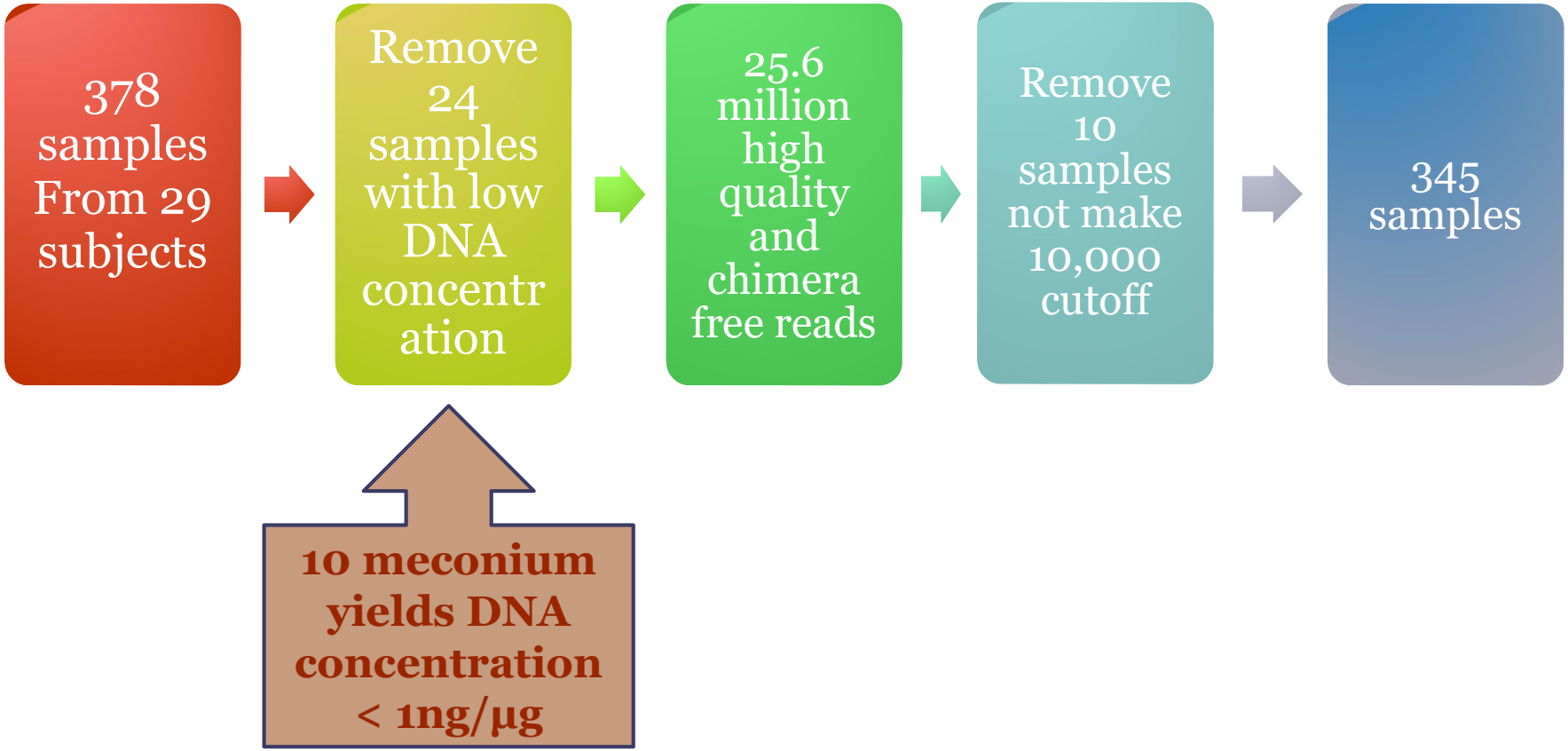
Results from First Cohort

Clinical characteristics of the initial 29 subjects:

		N (%)
Gender	Male	14 (48 %)
	Female	15 (52%)
Ethnicity	Hispanic	9 (31%)
	Non-Hispanic	20 (69%)
Delivery	Vaginal	12 (45%)
	C-section	16 (55%)
Race	White	22 (75 %)
	African American	5 (17%)
	Asian	1 (3%)

	Mean	SD
Gestational Age (wks)	31.3	1.7
Birth weight (g)	1460	445.3
SNAPEII	8.6	10.5

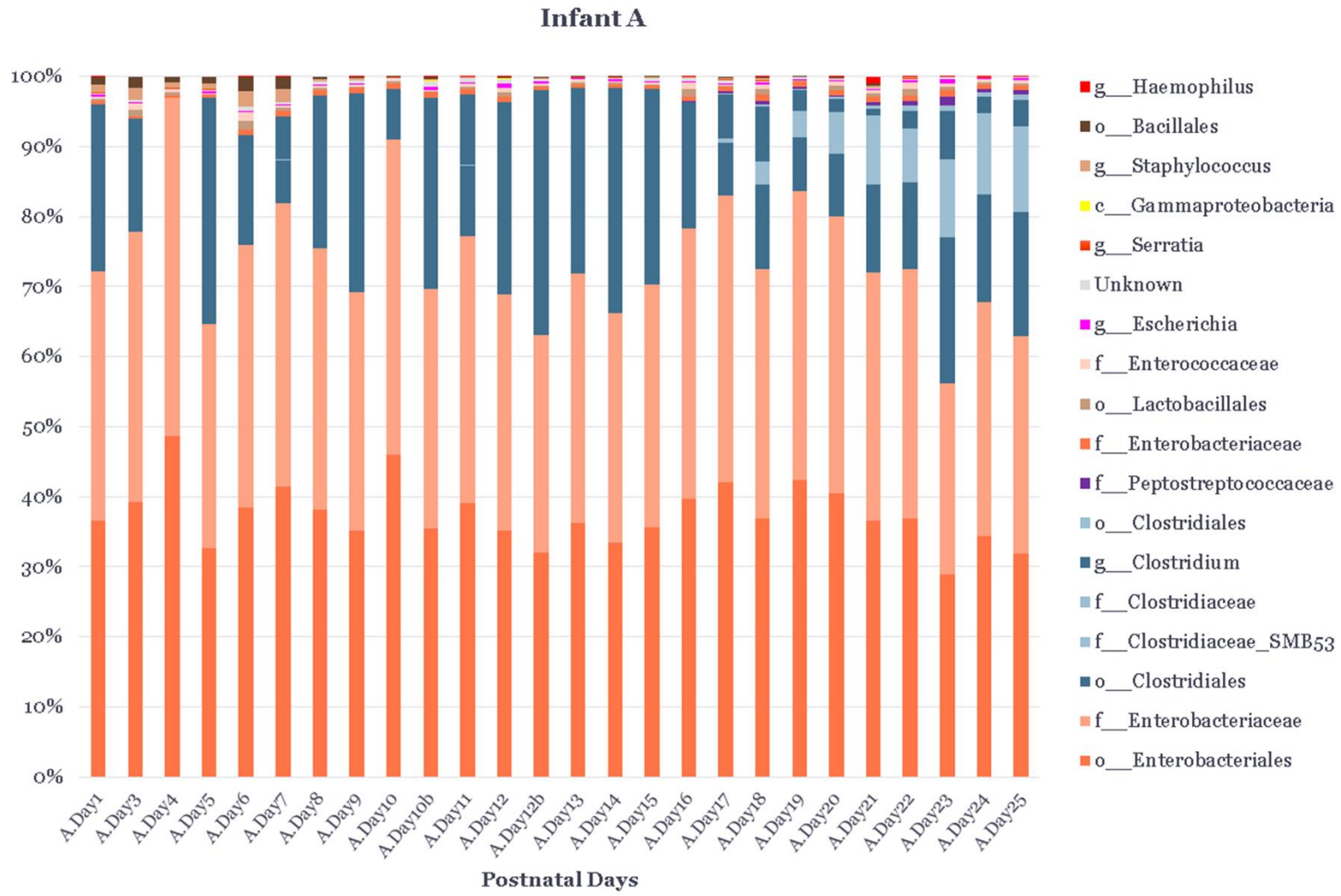
Workflow and Quality Control of Stool Samples

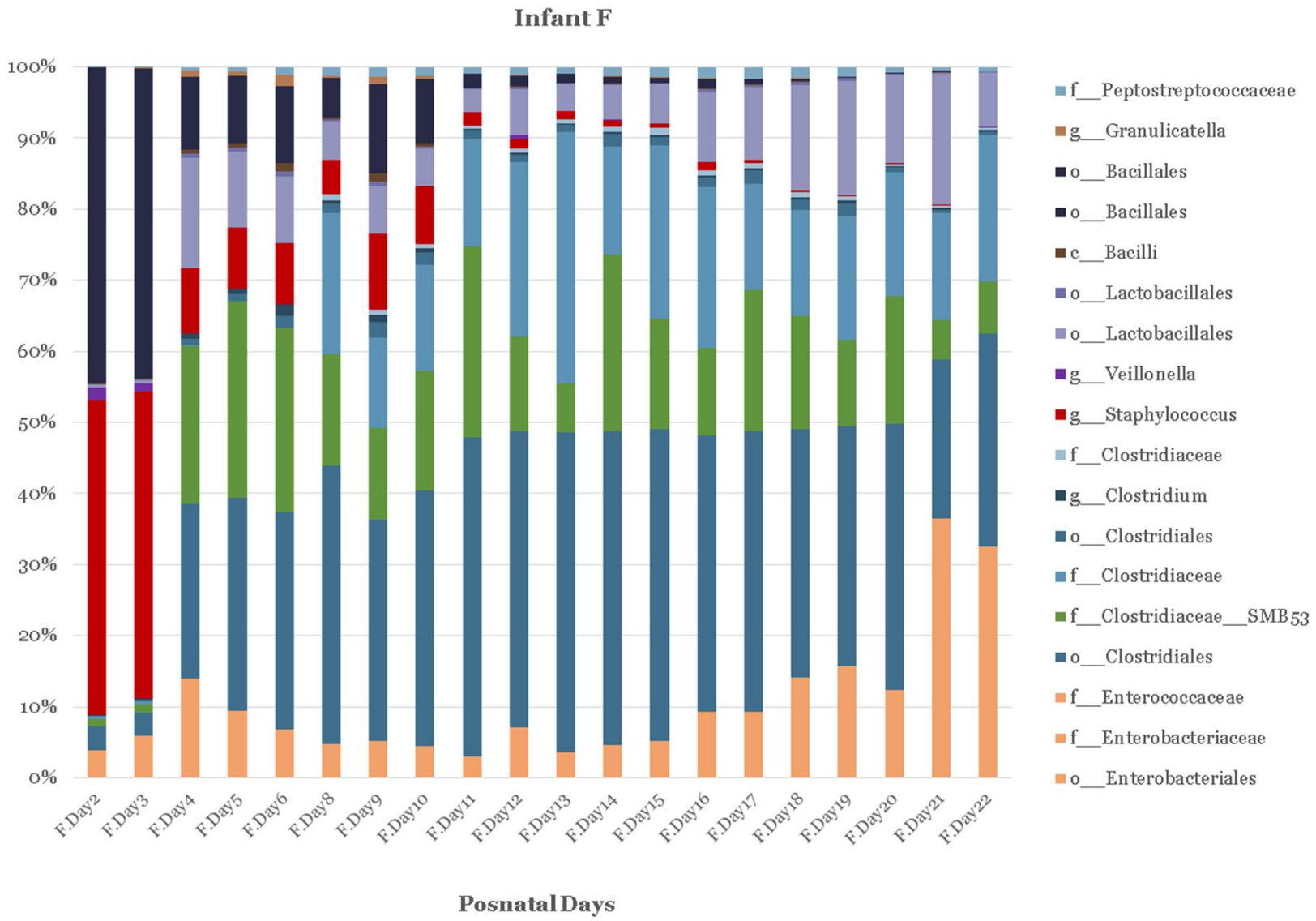


Result 1: Microbiome community composition in preterm infants

- The most abundant phyla:
  - Proteobacteria (54.3%)
  - Firmicutes (39.2%)
  - Bacteroidetes (3.9%)
  - Actinobacteria (2.4%).

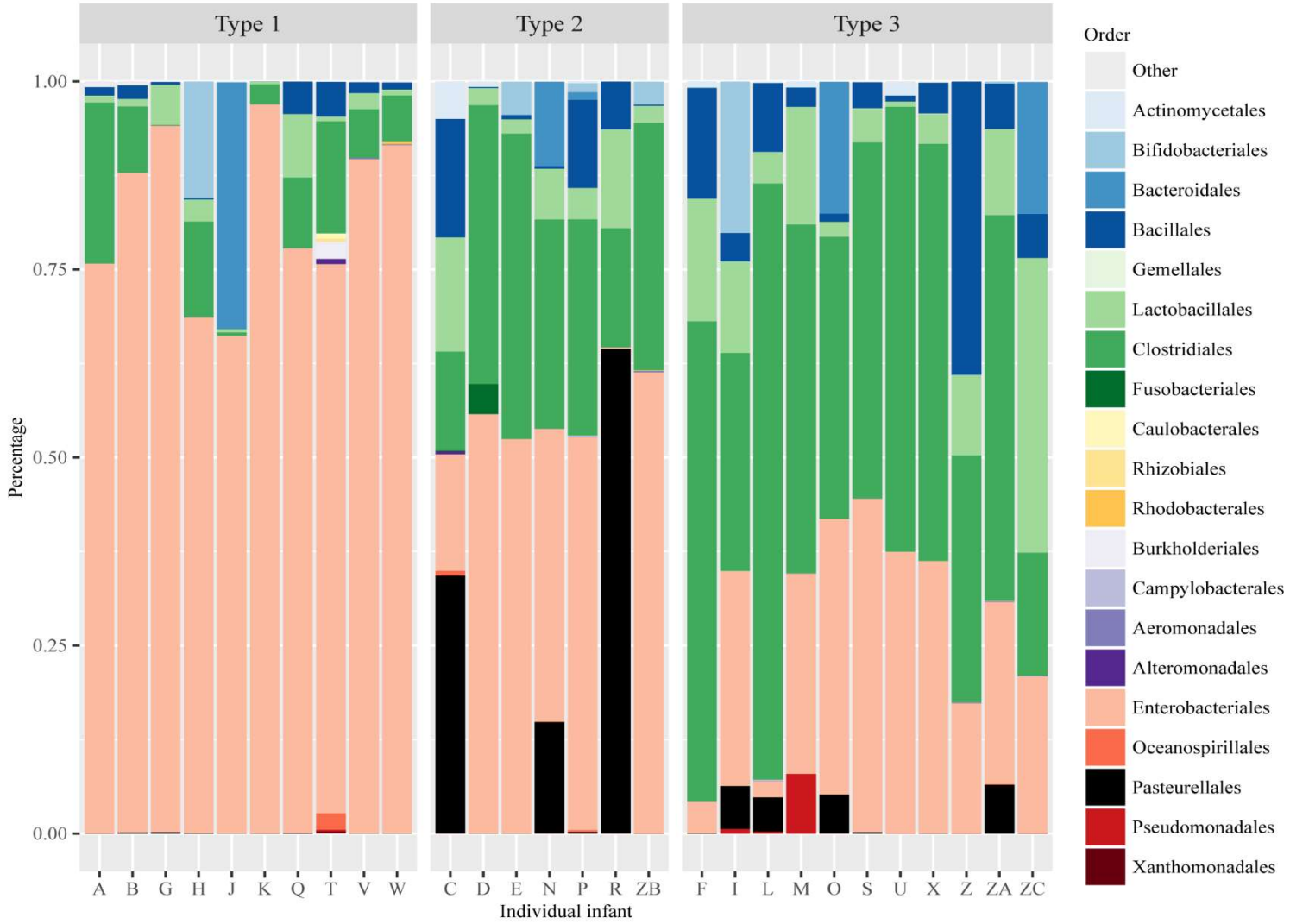
- What contributes to changes in the diversity of the microbiome (Linear mixed-effects models):
  - Time (postnatal days)
  - Gender
  - Feeding type (using mother’s breastmilk or not)


Infant A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
A.Day1
A.Day3
A.Day4
A.Day5
A.Day6
A.Day7
A.Day8
A.Day9
A.Day10
A.Day10b
A.Day11
A.Day12
A.Day12b
A.Day13
A.Day14
A.Day15
A.Day16
A.Day17
A.Day18
A.Day19
A.Day20
A.Day21
A.Day22
A.Day23
A.Day24
A.Day25
Postnatal Days
g__Haemophilus
o__Bacillales
g__Staphylococcus
c__Gammaproteobacteria
g__Serratia
Unknown
g__Escherichia
f__Enterococcaceae
o__Lactobacillales
f__Enterobacteriaceae
f__Peptostreptococcaceae
o__Clostridiales
g__Clostridium
f__Clostridiaceae
f__Clostridiaceae_SMB53
o__Clostridiales
f__Enterobacteriaceae
o__Enterobacteriales


Infant F
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
F.Day2
F.Day3
F.Day4
F.Day5
F.Day6
F.Day8
F.Day9
F.Day10
F.Day11
F.Day12
F.Day13
F.Day14
F.Day15
F.Day16
F.Day17
F.Day18
F.Day19
F.Day20
F.Day21
F.Day22
Posnatal Days
f__Peptostreptococcaceae
g__Granulicatella
o__Bacillales
o__Bacillales
c__Bacilli
o__Lactobacillales
o__Lactobacillales
g__Veillonella
g__Staphylococcus
f__Clostridiaceae
g__Clostridium
o__Clostridiales
f__Clostridiaceae
f__Clostridiaceae__SMB53
o__Clostridiales
f__Enterococcaceae
f__Enterobacteriaceae
o__Enterobacteriales

Gut Microbial Patterns by Individual Infants

Cong, Xu, Janton, Henderson, Matson, McGrath, Maas, Graf, (2016), PloS One



Result 2. Gut Microbiome and Feeding

Cong, X., Judge, M., Xu, W., Diallo, A., Janton, S., Brownell, E., Matson, A., Maas, K., Graf J. al. (in press) Influence of Infant Feeding Type on Gut Microbiome Development in Hospitalized Preterm Infants. Nursing Research

Kingdom

Phylum

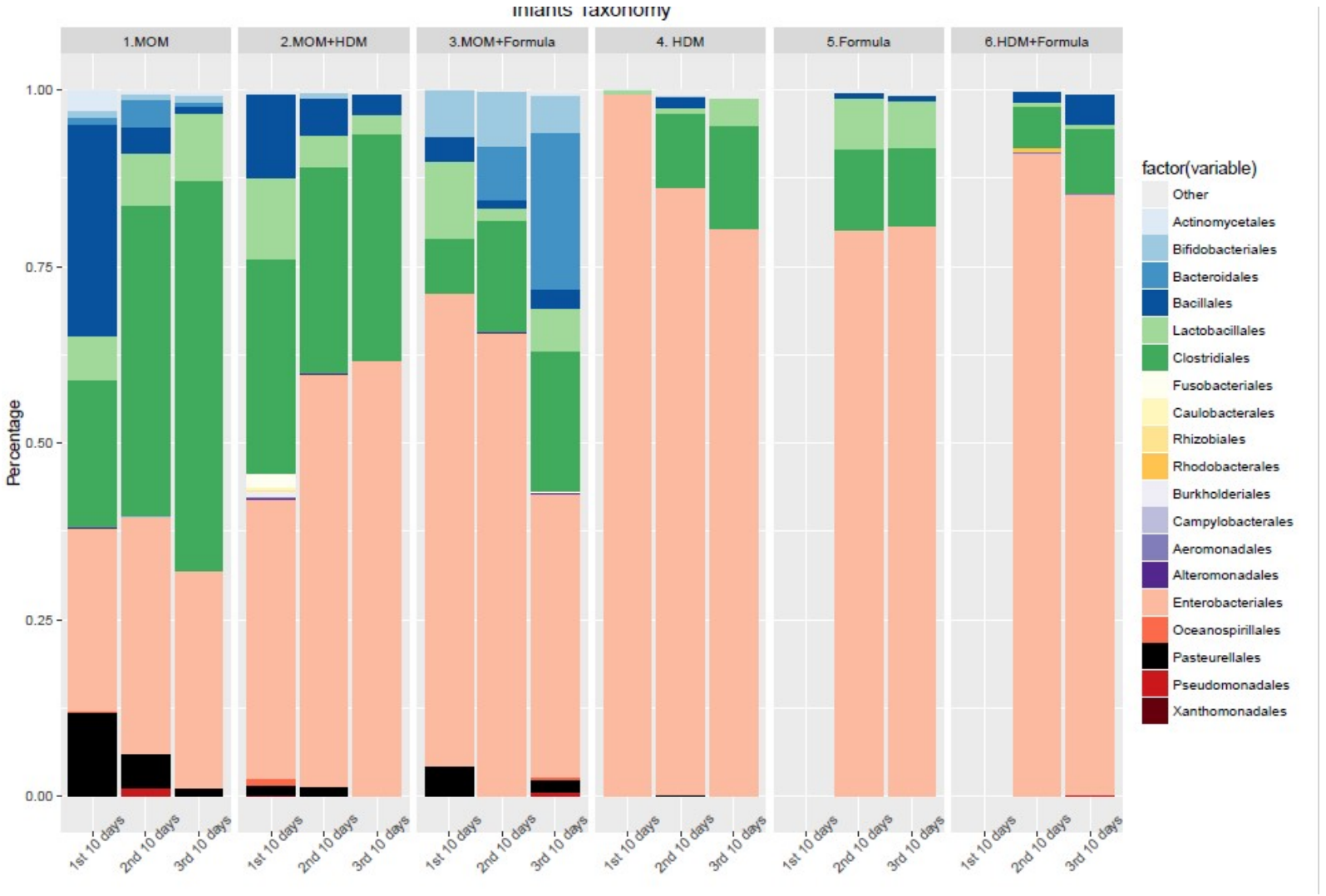
Class

Order

Family

Genus

Species



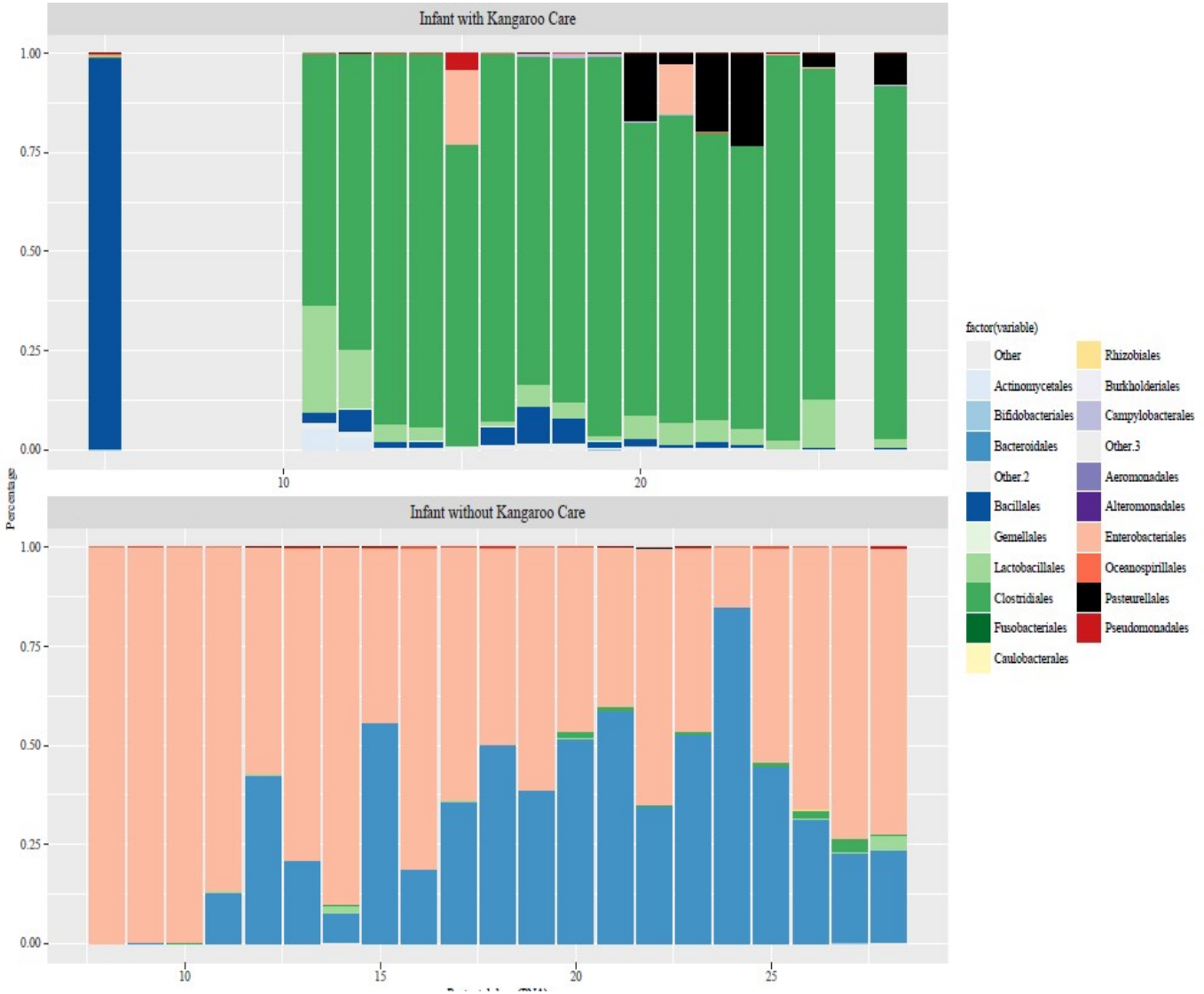
Result 3: Parent-Infant Contacts (Daily Average)

Data from the initial 50 subjects:

- KMC: 13.35 ± 32.10 min
- KPC (fathers): 1.44 ± 9.40 min
- Direct breastfeeding: 3.53 ± 13.41 min
- Other parent contacts
  - holding/cuddling: 28.61 ± 50.89 min
  - hand swaddling/touching: 14.15 ± 41.51 min
  - talking/singing/reading: 7.76 ± 33.04 min
- Total mother contacts: 53.80 ± 68.07 min
- Total father contacts: 16.73 ± 38.19 min

KMC and Gut Microbiome

- Infant with KMC (Subject 12) is 42.5% MBM and 57.5% formula; male
- Infant without KMC (Subject 10) is 98% MBM and 2%DBM; male



Result 4: Cumulative Pain/Stressors in the NICU

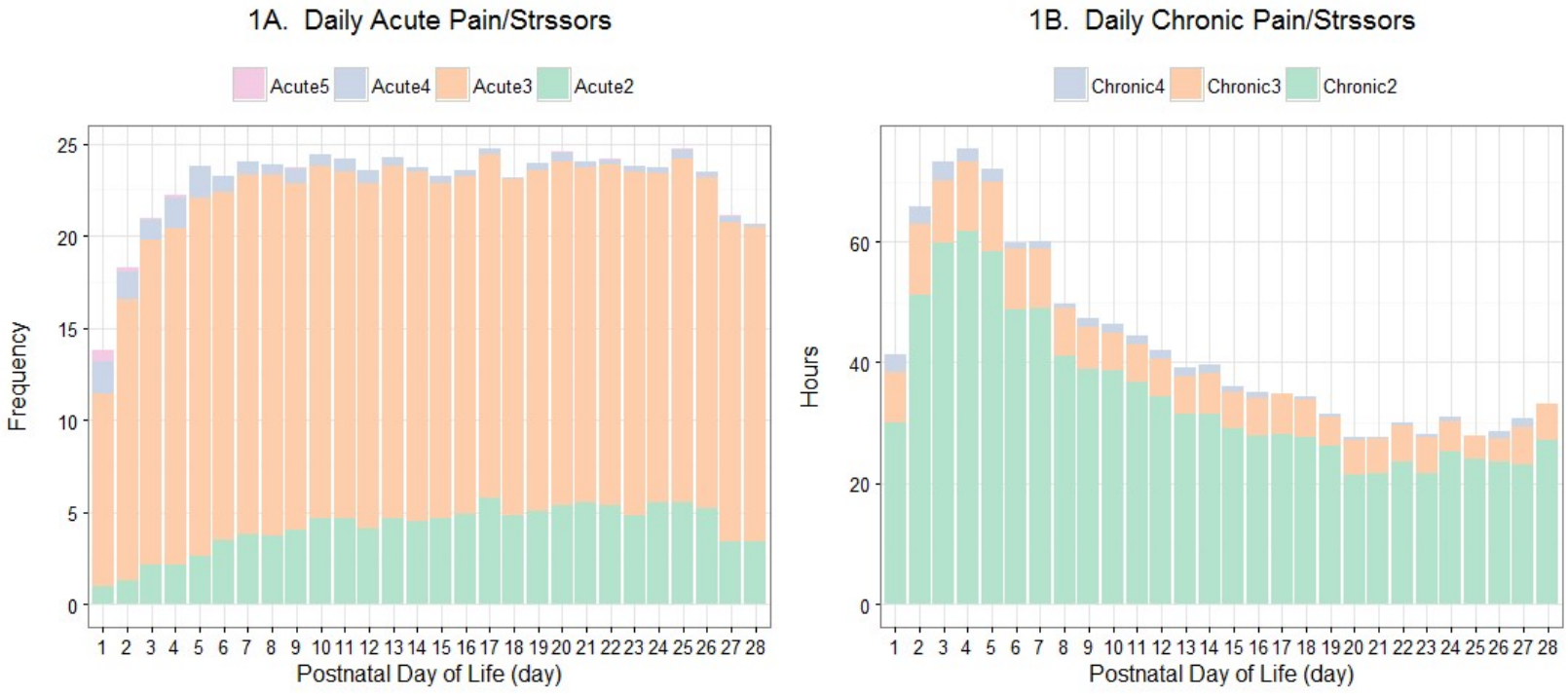
Data from initial 50 subjects:

- **Acute pain/Stressor** daily: 23.4 ± 7.2

  diaper change, heel sticks, arterial blood draw

- **Chronic procedures** daily 5.1 ± 3.2 hours

  PICC in situ, NG tube in situ, CPAP, systemic infection.



Result 5. Linear Regression Model of NNNS Subscales

	Stress/Abstinence (NSTRESS) N = 40				Habituation (NHABIT) N = 40		
Parameter	**Estimate**	**F-value**	**p-value**		**Estimate**	**F-value**	**p-value**
Gender Female	-0.0134	0.39	0.54		0.4027	0.86	0.39
Male	0.0000				0.0000		
Birth Gestational Age	-0.0090	0.82	0.37		-0.2275	-1.22	0.22
Last Body Weight	0.0462	1.47	0.23		-0.6975	-1.10	0.27
SNAPEII	-0.0003	0.03	0.85		-0.0059	-0.22	0.82
Delivery C-section	0.0369	1.10	0.30		-0.4879	-0.79	0.43
Vaginal	0.0000				0.0000		
Daily Acute NISS Score	0.0035	5.30	0.03*		-0.0889	-2.94	<0.01**
Daily Chronic NISS Score	0.0005	4.13	0.05		-0.0186	-2.48	0.02*
Daily Breastfeeding	-0.0003	0.03	0.85		0.0647	2.27	0.02*
Daily KMC Holding	0.0008	1.27	0.27		0.0670	4.81	<0.01**
	R-Squared		0.59		R-Squared		0.78

Note: *p<0.05, **p<0.01; Daily Acute NISS score = the daily weighted average of each severity level acute pain/stressors; Daily Chronic NISS score = the daily weighted average of each severity level chronic pain/stressors.

Linkages of KMC, Stressors, Gut Microbiome, and Neurobehavioral Outcomes

Indicator species of microbiota in different levels of pain/stressors experienced in the NICU

Phylum	Order / Genus	Indicator Value	Phylum	Order / Genus	Indicator Value
Infants Experienced Low level of *Acute* Pain/Stressors			**Infants Experienced Low level of *Chronic* Pain/Stressors**		
Actinobacteria	*Bifidobacteriales/Bifidobacterium*	0.65**	*Bacteroidetes*	*Bacteroidales/Bacteroides*	0.50*
Infants Experienced High levels of *Acute* Pain/Stressors			**Infants Experienced High level of *Chronic* Pain/Stressors**		
Firmicutes	*Lactobacillales/Enterococcus*	0.78**	*Firmicutes*	*Lactobacillales/Enterococcus*	0.85**
Firmicutes	*Lactobacillales/other*	0.72**	*Firmicutes*	*Lactobacillales/other*	0.80**
Firmicutes	*Lactobacillales/Granulicatella*	0.69**	*Firmicutes*	*Other/other*	0.77**
Proteobacteria	*Enterobacteriales/Pantoea*	0.52**	*Firmicutes*	*Lactobacillales/Granulicatella*	0.68**

Note: ** $p < 0.01$; * $p < 0.05$

Indicator species of gut microbiota with NNNS

Phylum	Order / Genus	Indicator Value
Infants with Less (better) NNNS-stress response		
Bacteroidetes	*Bacteroidales/Bacteroides*	0.55**
Infants with high (worse) NNNS-stress response		
Firmicutes	*Lactobacillales/other*	0.72**
Proteobacteria	*Enterobacteriales/Pantoea*	0.68**
Firmicutes	*Clostridiales/other*	0.56**
Proteobacteria	*Aeromonadales/other*	0.52**

Note: ** $p < 0.01$

Conclusions

- Over the first 30 days of early life, gut microbiome diversity begins low and increases daily after birth.
- Preterm infants' gut microbiome community is often dominated by Enterics.
- The amount of KMC and KPC and direct BF seemed not to meet preterm infants' needs.
- Preterm infants experience numeric acute and prolonged chronic painful/stressful procedures.
- Postnatal days of life, feeding, gender, and stress experience affect the composition of the gut microbiome.
- Gut microbiome may be omic markers of stressors for infant neurodevelopment.

Multidisciplinary Collaborations

Research Team:

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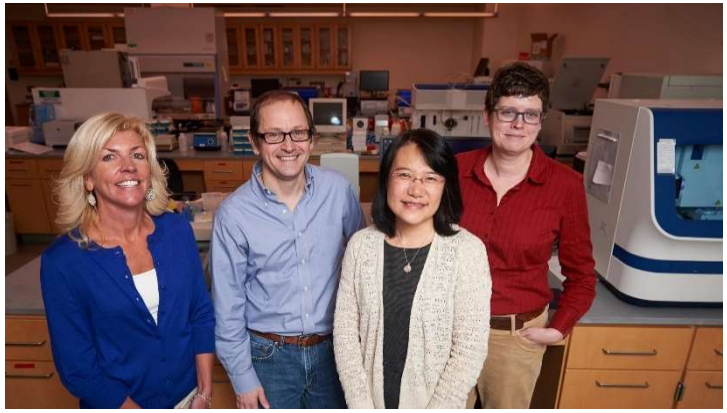
CT Children's Medical Center NICU Staff Members and Families



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