The gut microbiota and SES in preterm infants in the Chicago area

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Prematurity

- What is full term? 37-40 weeks gestation
- What is prematurity? < 37 weeks gestation
- What is the limit of viability? 22-23 weeks gestation
Prematurity

Full Term, 40 weeks

Premature, 23 weeks
Smallest Survivor

Birth weight 8.6 oz.
Survival vs Birth Weight for ELBW Infants

Survival (%)

Birth Weight (g)

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Day of Death for ELBW Non-Survivors

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Survival as a function of birthweight for all patients alive on Day 4 (n = 249)

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The Preterm Infant - 12% of births

Disproportionately account for:
- 40% of children who have cerebral palsy (CP)
- 25% of children with hearing impairment
- 35% of those with vision impairment.
The NICU

- Antibiotics
- Breast Milk vs. Formula
- Opioids
- Instrumentation
- NICU
- Delayed Feeding
- H2 Blockers
Like the canary in the coal mine—or asthmatics in air pollution studies—children born preterm may serve as a sentinel population owing to increased susceptibility to the sometimes modest effects of common toxicants, improving study power and decreasing necessary sample size.
Necrotizing Enterocolitis (NEC)-

Inflammatory bowel necrosis that primarily afflicts premature infants after the initiation of enteral feeding.

Risk Factors

- Prematurity
- Bacterial Colonization
- Enteral Feeding
- Hypoxia/Altered intestinal blood flow
NEC and Neurologic Outcome

Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight Infants after NEC Hintz, et al Pediatrics 2005

Multicenter, retrospective analysis 1995-1998
Infants in NICHD Neonatal Network <1000gm
5553 ELBW entered into registry
2948 infants evaluated at 18 and 22 months
124 – surgically managed NEC
121 – Medically managed NEC
Neurodevelopmental Outcome associated with NEC

<table>
<thead>
<tr>
<th>Condition</th>
<th>SurgNEC</th>
<th>MedNEC</th>
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<tbody>
<tr>
<td>Cerebral Palsy</td>
<td></td>
<td></td>
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<tr>
<td>MDI&lt;70</td>
<td></td>
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<tr>
<td>PDI&lt;70</td>
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<tr>
<td>NDI</td>
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</tr>
<tr>
<td>PVL</td>
<td>14%</td>
<td>7%</td>
</tr>
<tr>
<td>BPD</td>
<td>57%</td>
<td>43%</td>
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<tr>
<td>CP</td>
<td>24%</td>
<td>15%</td>
</tr>
<tr>
<td>Decreased growth</td>
<td>all parameters</td>
<td></td>
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</tbody>
</table>

Hypothesis:

Enteral feeding results in colonization of the uniquely susceptible premature intestine with pathogenic bacteria, resulting in an exaggerated inflammatory response.
Question:

• Can microbiome analysis be used to identify the pathogenic bacteria associated with NEC?

Methods:

• 20 patients - 10 with NEC 10 control
  • 4 sets of twins
• Analysis of fecal samples prior to onset of NEC by 16S rRNA sequencing
Bacterial Diversity and NEC

Bacterial Colonization and NEC

Bacterial Colonization and NEC
Shift in Microbiome

Claud et al. Microbiome, 2013
Temporal progression of the preterm infant microbiota

Claud et al. Microbiome, 2013
Modification of the early microbiome and NEC

Absolute risk difference .03
NNT - 32

Prematurity

Full Term, 40 weeks

Premature, 23 weeks

Host development coincides with microbiome development
Growth in the Neonatal Intensive Care Unit Influences Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight Infants

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CONCLUSIONS. These analyses suggest that growth velocity during an ELBW infant’s NICU hospitalization exerts a significant, and possibly independent, effect on neurodevelopmental and growth outcomes at 18 to 22 months’ corrected age.
Preterm Infant Microbiota ($M_{PI}$)

![Graph showing weight gain and microbiota composition over weeks of life for $M_{PI}$-H and $M_{PI}$-L groups.]

**$M_{PI}$-H**

**Good weight gain**
- Pyrosequencing ID
  - Comamonas sp: 21
  - Weissella confusa: 13
  - Leuconostoc citreum: 10
  - Comamonas testosteroni: 10
  - Lactococcus sp: 10
  - Acinetobacter sp: 3
  - Citrobacter sp: 3
  - Lactococcus lactis: 3
  - Delftia sp: 2
  - Polaromonas sp: 2
  - Alcaligenes sp: 2
  - Enterococcus italicus: 1
  - Streptococcus sp: 1
  - Chryseobacterium bovis: 1
  - Lactococcus raffinolactis: 1
  - Aeromonas sp: 1
  - Arcobacter butzleri: 0.5
  - Brevundimonas sp: 0.5
  - Fusobacterium sp: 0.5
  - Bacteroides sp: 0.5
  - Veillonella sp: 0.5
  - Weissella cibaria: 0.5

**$M_{PI}$-L**

**Poor weight gain**
- Pyrosequencing ID
  - Comamonas sp: 16
  - Weissella confusa: 15
  - Leuconostoc citreum: 12
  - Lactococcus sp: 12
  - Comamonas testosteroni: 11
  - Citrobacter sp: 6
  - Delftia sp: 4
  - Lactococcus lactis: 4
  - Acinetobacter sp: 3
  - Streptococcus sp: 2
  - Enterobacter sp: 2
  - Lactococcus raffinolactis: 1
  - Leuconostoc sp: 1
  - Flavobacterium sp: 1
  - Stenotrophomonas maltophilia: 1
  - Ralstonia sp: 0.5
  - Moraxella osloensis: 0.5
  - Veillonella sp: 0.5
  - Pseudomonas sp: 0.5
  - Acidovorax sp: 0.5
  - Achromobacter sp: 0.5
  - Weissella cibaria: 0.5
  - Kordia sp: 0.5
Transfer of infant microbiome to germ free mice

Fecal lysate from infant

Germ free pregnant dam C57/Bl6

Litter

Daily weight

Wean
Inflammatory Response Related Networks

$M_{PL\_L}$

$M_{PL\_H}$
Relative mRNA expression levels in M<sub>PI</sub>-L and M<sub>PI</sub>-H ileum
Cytokine Expression

Serum

Cytokine unit/0.1ml serum

- IL-1β
- IL-4
- IL-6
- IL-10
- IL-18
- IFN-γ
- TNFα

GF – GermFree

Mπ-L

Mπ-H

SPF

GM-CSF
NF-κB Activation

Labeling index for pNFkB p65 nuclear translocation
NF-κB Dependent Cytokines

GF

M_{\text{pl}}-\text{L}

M_{\text{pl}}-\text{H}

SPF

VCAM-1

Magnification 40x

MCP-1
Inflammation and Prematurity

NEC

BPD

PVL

ROP
Neurodevelopmental Outcome in Preterm infants

Morbidities
1. Bronchopulmonary dysplasia
2. Necrotizing Enterocolitis
3. Intraventricular Hemorrhage
4. Periventricular Leukomalacia
5. Retinopathy of Prematurity
6. Sepsis

Neurodevelopmental Impairment at Age 2

Cumulative morbidities for ELBW infants at NICU discharge
Regulation of cortex neuronal development by gut microbiota.

A.

<table>
<thead>
<tr>
<th>SPF</th>
<th>GF</th>
<th>M_{PL-L}</th>
<th>M_{PL-H}</th>
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<tr>
<td>1</td>
<td>2</td>
<td>3</td>
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- **NeuN**
- **GAPDH**

B.

- **SPF**
- **GF**
- **M_{PL-L}**
- **M_{PL-H}**

**DAPI**

**NeuN**
Regulation of cortex myelination by gut microbiota.

A.

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<td>3</td>
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</tbody>
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MBP

GAPDH

B.

DAPI

MBP

SPF

GF

$M_{\text{PI-L}}$

$M_{\text{PI-H}}$
IGF1

- We have established that certain microbiota colonization normalized the growth in GF mice (M2).

- Mutation(s) in the \textit{igf-1} gene or in the \textit{igf1r} gene are found to be associated with severe body growth failure, microcephaly, and developmental delay.

- In rodents, \textit{igf-1} gene disruption results in reduced brain size, CNS hypomyelination and loss of hippocampal granules and striatal parvalbumin-containing neurons.

- GF mice have lower circulating IGF-1 comparing to SPF mice.

- IGF1 crosses the blood brain barrier
Hypothesis

• Microbial colonization can modulate brain development through regulation of IGF1.
Microbiome influences serum IGF-1

A

Serum IGF-1 2 weeks

B

Serum IGF-1 4 weeks
Alterations in brain IGF-1
Alterations in brain IGF1r and IGFBP3
Effect of socioeconomic status on Neurodevelopment


Microbiome?
The microbiome as a potential mediator of socio-economic disparities in preterm infant neurodevelopmental trajectories from NICU discharge to school age

The means by which poverty alters neurodevelopment are unknown. The microbiome is influenced by environment and in turn influences brain development.

We hypothesize that the microbiome is a biologic effector of the influence of SES and environment on neurodevelopment.
Influences on Preterm Infant Neurodevelopment Potential From Birth To School Age

**BIRTH**
- Probability of neurodevelopment impairment based on birth GA alone, 30%

**NICU**
- EPOCH 1
  - Birth GA, e.g. 25 weeks
- EPOCH 2 - AIM 1
  - NICU morbidities + Early microbiome

**HOME**
- Disadvantaged SES Home Environment after NICU Discharge
- Advantaged SES Home Environment after NICU Discharge

**SCHOOL**
- Higher Probability Of Impairment
  - School Readiness
- Lower Probability Of Impairment

- EPOCH 3 – AIM 2
  - Post-discharge environment + Infant microbiome - AIM 3
The Microbiome is Modifiable
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