Normal neonatal microbiome variation in relation to environmental factors

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Objectives

- Describe the developing neonatal microbiome in relationship to varied environmental factors
  - Mode of delivery
  - Environmental toxins
  - Antibiotic exposure
  - Breastfeeding and diet
Objectives

• Discuss potential implications for short-term and long-term health
  – Allergy/atopy
  – Infection
  – Obesity
  – Cancer, inflammatory bowel disease, cardiovascular disease
The complexity of the human microbiome

Program Snapshot

The Common Fund’s Human Microbiome Project (HMP) aims to characterize the microbial communities found at several different sites on the human body, including nasal passages, oral cavities, skin, gastrointestinal tract, and urogenital tract, and to analyze the role of these microbes in human health and disease. HMP includes the following initiatives.
Intestinal microbiome: health & disease

• Described as co-evolving with humans, creating a metabolic “superorganism”  
  Dethlefsen et al, Nature 2007

• Critical for:
  • immune maturation
  • drug metabolism
  • energy metabolism  
  Lederberg, Science 2000

• New diseases associated including obesity, IBD, circulatory diseases  
  Kinross et al, Genome Med 2011
The microbiome: meaningful patterns?

• One larger metagenomic compilation study identified a core microbiome and specific “enterotypes” Arumugam et al. Nature 2011

• Endless possibilities for potential targeted therapies to alter the microbiome to treat disease or enhance health Nicholson et al, Science 2006.
Maslowski KM, Mackay CR.
Ongoing projects

• **Premature infant cohort**
  - Evaluating the developing gut microbiome in prematurity as it relates to sepsis

**Healthy infant controls**

• **CF infant/child cohort**
  - Evaluating the developing gut and lung microbiome in CF as it relates to:
    - Medical and dietary interventions
    - Pseudomonas colonization
    - CF exacerbations and growth failure
    - ΔF508 homozygosity

• **The Children’s Center at Dartmouth**
  - Evaluating the developing gut microbiome in healthy infants over the first year of life with investigation of effects of environmental toxins and infection risk
Intestinal microbial richness and variation

Inter-subject variability

\[ ICC = \frac{D_{11}}{D_{11} + \sigma^2} \]

Inter- plus intra-subject variability

<table>
<thead>
<tr>
<th>Color</th>
<th>Genera</th>
<th>Proportion of total number of reads</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteroides</td>
<td>18%</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Bifidobacterium</td>
<td>12%</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Veillonella</td>
<td>10%</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Clostridium</td>
<td>9%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Blautia</td>
<td>9%</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Parabacteroides</td>
<td>8%</td>
<td>0.60</td>
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<tr>
<td></td>
<td>Streptococcus</td>
<td>6%</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Lachnospira</td>
<td>3%</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Microbial diversity is increasing in both systems

- Diversity was assessed using the Simpson’s Diversity Index (SDI)

  • Increasing trend in microbial diversity for both the respiratory (\(P < 0.001\)) and intestinal tracts (\(P = 0.037\))

  • Diversity in the respiratory increasing at a faster rate (\(P = 0.070\))

Madan et al *Mbio* 2012
Effect of exposures on diversity trajectories

- Breastmilk
- Formula

- H2 antag
- No H2 antag

Madan et al Mbio 2012
Characterizing the effect of exposures on respiratory microbiome development

- H2 blockers: $p = 0.057$
- Solid food: $p = 0.28$
- Breastfeeding: $p = 0.01$
- Antibiotics: $p = 0.89$

Madan et al. *Mbio* 2012
Normal neonatal microbiome variation in relation to environmental factors
Neonatal microbiome

- sterile at birth
- Shaped over time by multiple exposures
- complex interplay
  microbiome  immune system
Neonatal microbiome

• Defining a ‘healthy’ microbiome in the neonatal and infant period is more accessible with culture-independent technologies

• Differences in the microbiome as they relate to short term and long term disease highlight opportunities for intervention and disease prevention
Neonatal colonization

• Until recently relied upon culture based or targeted molecular studies → incomplete
  Palmer et al Plos Biol 2007

• Complete investigation using massively parallel deep sequencing permits unbiased analysis of patterns
  Jacquot et al J Pediatr 2011

• Establishing symbiotic bacteria can act as a central stimulus for maturation of the immune system and alter risk for disease
  Backhed F. Clin Exp Immunol 2010
Culture-Independent technologies allow for complete sampling

- FISH
- DNA pyrosequencing
- Microarrays (Phylochip)
- QT PCR

PLUS

- Complex bioinformatics techniques for massive data
Human microbiome

• Species vary significantly between people
• Species vary significantly between body habitat
• Gut: Bacteroidetes and Firmicutes (Phyla)
• Anchor microbes? Or a human core \cite{Amururgam:2010}
• Infant phyla predominately Firmicutes, Bacteroidetes and Proteobacteria
• *High species variability in infants* \cite{Morowitz:2011}
Neonatal microbiome composition (breastfed infants)

- Initially facultative aerobes:
  - *Staph*
  - *E Coli*
  - *Enterococcus*
- Then anaerobes
- Then Human Milk Oligosaccharides arrive and shift to anaerobes:
  - *Bifidobacteria*
  - *Bacteroides*
  - *Clostridium* Johnson et al Pediatr 2012
Mode of delivery
Delivery mode

- Fetal life essentially sterile
- Birth presents first encounter with microorganisms that rapidly populate the gut
- Study of vaginal vs. c-section birth suggests that the primary assemblages are dictated by delivery mode
Vaginal birth

• These infants are more likely to become colonized by organisms comprising maternal vaginal microbiome Dominguez-Bello et al PNAS 2010
  – *Lactobacillus*
  – *Prevotella*
Pregnancy and microbiome

• Study of 84 women during pregnancy found vaginal microbiome undergoes a decrease in diversity

• Becomes enriched with *Lactobacillus* species

• May relate to the vertical transmission that occurs at birth  Aargaard K et al. PLoS One 2012
Operative delivery

- These infants are more likely to be colonized with bacteria present on mother’s skin and from the hospital environment
  - *Staphylococcus*
  - *Propionibacterium*
  - *Corynebacterium*
Delivery mode shapes microbiota across multiple body sites

Delivery mode impacts microbiome long term

• In a longitudinal study of 605 infants from 5 European countries
• Repeat profiling of the microbiome at 6 weeks and post weaning found that
  – Mode of delivery
  – Feeding method (breast versus formula)
• Had *persistent effects* on microbial composition  
  Fallani M et al. Micro 2011
Public health issue?

• If early shifts in the development of the microbiota related to c-section have lasting health consequences...
• This might impact a substantial number of children
• 30% of births in the US occur by c-section  CDC report 2012
• Infants delivered by c-section have higher risk of MRSA infection  Gregory KE Neon Nurs 2011
  – Related to pioneer colonizers?
  – Or related to lack of protection against pathogens by vaginally transmitted microflora?
Environmental toxins
Environmental toxicants

- Microbial transformations may increase the bioavailability of nutrients
- These same processes can produce more toxic forms of contaminants
- Van de Wiele et al showed that normal human intestinal bacteria metabolize environmental contaminants
  - Turn polycyclic aromatic hydrocarbons into bioactive estrogen-like molecules
  - Transform metals into volatile toxic products
  - Affect species balance and function = dysbiosis

Van de Wiele et al Environ Health Perspect 2005
Heavy metals

• Growing presence of antibiotic resistant microbes in non-antibiotic exposed young children
• Due to widespread contaminants from food and environment
• Mercury exposure results in resistance to multiple antibiotics  Skurnik D et al J Med Microbiol 2010
Arsenic

• Fetuses, infants and children are regularly exposed to arsenic through well water and foods (rice and baby formula) 
  Jackson BP et al. Environ Health Perspect 2012

• Metals, like arsenic, used historically as an antibiotic and is currently added to animal feed routinely (chicken feed)

• Leading to emergence of metal/antibiotic coresistance strains in livestock, including MRSA 
Antibiotics
Perinatal and early life antibiotic use

- The average child in the US is exposed to 10-20 courses of antibiotics before their 18th birthday
- Perinatal and early life antibiotic use as well as infectious diseases →
  - influence the establishment of microbial communities
  - Cause large shifts in bacteria & alter diversity

Koenig JE et al PNAS 2011
Infants and antibiotics

- Study of 31 amoxicillin treated infants with ARI and look at *Bifidobacterium* effects
  - Mangin et al. Anaerobe 2010
- Complete elimination of *Bifidobacterium adolescentis*
- Significant decrease in *Bifidobacterium bifidum*
- No changes in overall counts of *bifidobacterium* at the genus level → profound shifts on the species level
High risk neonates and antibiotics

• NICHD studies have shown empiric antibiotic use is associated clearly with increased risk of
  – Necrotizing enterocolitis
  – Sepsis  Cotten et al Pediatrics 2009

• When evaluating fecal samples from antibiotic exposed infants later in infancy
  – Antibiotic resistance reduced
  – Overall diversity increased  Fallani et al Microbiol 2011
Antibiotics and obesity risk

- Potentially long term effects on the developing microbiome from early life antibiotic exposure
- Association between childhood overweight and obesity and early life antibiotic use

- 28,000 mother infant dyads studied
- 50% increased risk of overweight in children at age 7 if exposed to antibiotics <6 months
Antibiotics and drug resistance

  – Is maternal transmission possible?

• Attributed to environmental exposures
  – Foods
  – Mercury
  – Arsenic
Breastfeeding and the microbiome
Diet

• Breast fed infants have lower levels of potential pathogens:
  – *Clostridium difficile*

• Breastfeeding is associated with lower risk of childhood and adult onset obesity
  Thompson AL et al Am J Hum Biol 2012

• This is due in part to microbiome effects as early diet guides colonization
Diet

• Bacteria possess varying abilities to extract nutrients and energy from food

• The microbiome can shift an infant’s energy storage potential  
  Zivkovik AM et al PNAS 2011

• Oligosaccharides in breastmilk:
  – Promote *bifidobacterium* growth in the gut  
    Zivkovik AM et al PNAS 2011
Diet

• Study of 30 children enrolled in a longitudinal study found that:

• At age 10, overweight children had lower levels of bifidobacterium as infants compared with their normal weight counterparts

Luoto R et al J Pediatr Gastroenterol Nutr 2011
Diet

• Infants fed formula had higher proportions of
  – *Bacteroides*
  – *Prevotella*  Holscher et al JPEN J Parenter Enter Nutr 2012
  – *Clostridia difficile (associated with eczema)*

• Transition from:
  – breast milk to formula,
  – introducing solid foods,

influence the bacterial succession  Fallani et al Microbiol 2011
The microbiome and outcomes
Pediatric diseases associated with the microbiome

• NEC
• Sepsis
• Allergy/atopy
• Obesity
• IBD/Crohn’s
• ?psychiatric illness?
• Later cancer risk and cardiovascular disease
Allergy and atopy

• Germ free animal studies
• Absence of microbial colonization results in altered gut epithelialization, growth and immune function
• Specific bacteria in the gut associated with early onset allergy and atopy
  – *C diff and E coli* Sepp et al Clin Exp Allergy 2005
Neonatal microbiome and allergy

- Decreased microbial diversity in the *first weeks of life* related to allergy at school age (Wang et al J Clin All Imunol 2008; Bisgaard H et al J Allergy Clin Immunol 2011)

- Infants exposed to antibiotics, microbes influence maturation of Th1 immune responses (Oyama et al J Clin Immunol 2011)

- Enhanced maturation of protective immunoglobulins noted with *Bacteroides fragilis*
Microbiome and host transcriptome

- A novel evaluation of diet-dependent interactions
- Host transcriptome and microbiome
- Identified differences in bacteriology between breastmilk and formula at 3 months
- Differences in mRNA related to:
  - metabolism,
  - immunity,
  - defense genes,
  - which were upregulated in breast fed infants

Schwartz et al, Genome Biol 2012
Infection

• A direct link between gut colonization and infection has been described in high risk populations

• Premature infants' intestines are colonized with pathogens (maternal infection and infant antibiotic exposure)

• Life threatening infection linked to pioneer colonizers and lack of diversity in premature infants

Madan et al Arch Dis Child 2012
Future research

• Neonatal microbiome might dramatically influence lifelong health and disease risk
• Culture independent techniques more accessible
• Epidemiological studies to define ‘healthy’ developing microbiome in infancy and the impact of specific exposures are critical
Future research

• Studying the link between maternal, fetal, neonatal microbiome to identify preventable causes of preterm delivery and fetal basis of disease

• Potential translation to clinical work:
  – Informing newborn delivery mode
  – Breast milk benefits
  – Limiting or altering antibiotic exposure
  – Targeted probiotics
  – Individualization of medication regimens based on microbe profiles
Summary

• Studying “normal” patterns of colonization and links with disease are in their infancy
• Certain exposures may have profound effects on the microbiome in early life
  *many of which can be modified*
• Future epi studies in large populations will be extremely informative as we strive to define a healthy microbiome and ameliorate disease risk
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