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Microbiome Dysbiosis: An Emerging Consideration in the Pathophysiology of Inborn Errors of Metabolism

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Disclosures

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- ***This does not pose a conflict of interest for this presentation***

The opinions reflected in this presentation are those of the speaker and independent of Nutricia North America

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Learning Objectives

- Understand dysbiosis and evaluate current research on dysbiosis in IEM.
- Define the gut-brain axis and identify how dysbiosis impacts disease presentation.
- Outline the role pre-, pro-, and synbiotics might play in the therapy of dysbiosis.

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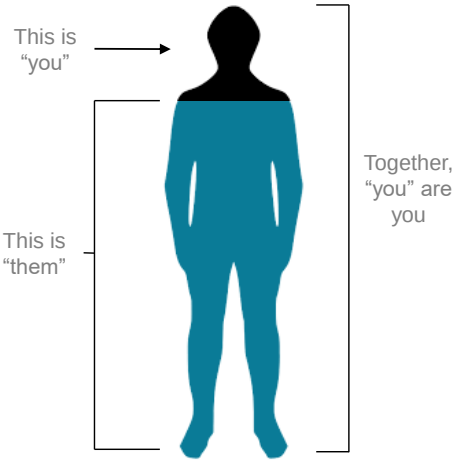
The Human Microbiome

- A highly complex and biochemically diverse community
  - Microbes compose ~90-60% of total cell composition[1]
  - Microbial genes represent ~99% of total unique gene composition[2]
    - Highlights the significant role microbiota play in human health



1. Sender, et al. PLoS Biol. 2016;14(8):e1002533. **2.** Qin, et al. Nature. 2010;464(7285):59-65.

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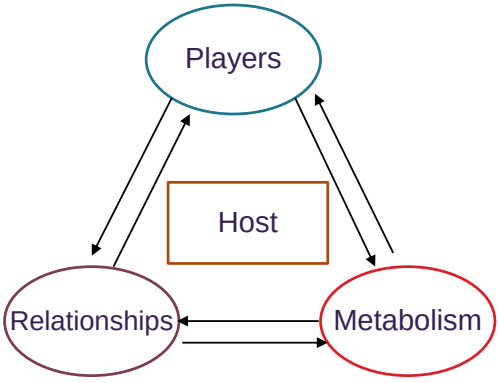
What Is a Microbiome?

- The collection of microbes, their genes, their metabolites, and totality of symbioses
  - Microbes
    - Bacteria
    - Fungi, phages, others
  - Metabolism
    - Produced **and** consumed compounds
  - Symbioses
    - Relationships between every player



Kirby, et al. Mol Genet Metab. 2020;131(1):1-10.

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What Is a Healthy Gut Microbiome?

- Depends on the individual
  - Structural trends
    - Dependent on lifestyle factors
      - Diet, cultural habits, exercise, stress, weight, antibiotic usage, etc.
  - Diversity
    - Stabilizes ~3 years of age
    - Decreases in elderly population (~70+ years of age)

Rinninella, et al. Microorganisms. 2019;7(1):14.

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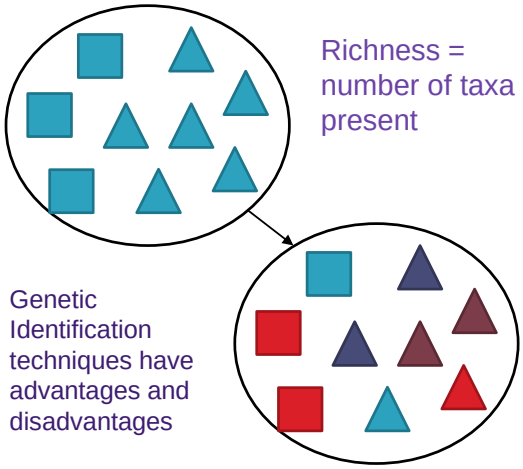
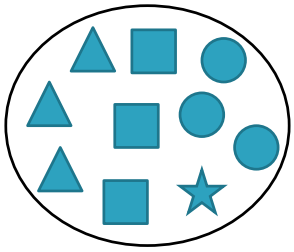
What is Diversity?

- What alpha diversity means with the microbiome…



Richness = number of taxa present

Genetic Identification techniques have advantages and disadvantages



Evenness = equal representation of each taxa

The two most common techniques are **16S ribosomal DNA** and **Whole Genome Sequencing**

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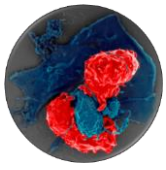
What Is a Healthy Gut Microbiome? (cont.)

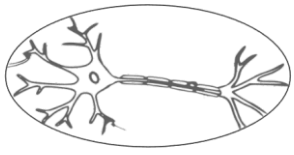
- Healthy = structurally stable and diverse
  - Functionally robust
- Stability is critical
  - Many challenges will present themselves

Inflammation



Healthy microbiota regulates proper immunological responses

Homeostasis



Healthy microbiota regulates nerve cell function

Pathogenic Defense

Spatial competition and defense against pathogens

1. Pandiyan. et al. Front Immunol. 2019;10:426. **2.** Strandwitz. Brain Res. 2018;1693:128-33.

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Dysbiosis – The Unhealthy Microbiome

- Eubiosis = balance; Dysbiosis = imbalance
  - Diversity typically is lowered in dysbiotic states
  - Relative to the individual and condition/disease
  - Extent of dysbiosis can correlate with disease
    - Neurological Disorders[1]
    - Endocrine Disorders[2]
    - Inborn Errors of Metabolism (IEM)???

1. Agorastos, Bozikas. Psychiatriki. 2019;30(3):189-92. **2.** Liu, et al. Cell Metab. 2020;31(1):77-91.

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What do we know about dysbiosis and IEM?

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Before We Get Started…

- Important to consider and remember:
  - Everyone has a unique microbiome
  - Dietary intervention depends on the individual
  - Disease presentation can vary
- Trends give us insights – theoretical associations
  - Specific shifts in taxa?
  - Alterations in diversity?
  - Functional shifts?

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Overview

IEM	Experimental Population(s)	Citations
PKU	n = 8, age = 4.24 ±2;	Pinheiro de Oliveira, et al. PLoS One. 2016;11(6):e0157513.
	n = 11, age = 33 ± 2;	Mancilla, et al. Microorganisms. 2021;9:530.
	n = 21, age = 10.0 ± 4;	Bassanini, et al. Front Cell Infect Microbiol. 2019;9:101. Verduci, et al. Nutr Metab Cardiovasc Dis. 2018;28(4):385-92.
HCU	n = 6, age = 26 (median)	Rizowy, et al. Biochimie. 2020;173:3-11.
GSD	n = 24, age = 10 to 20	Colonetti, et al. PLoS One. 2019;14(4):e0214582.

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Phenylketonuria (PKU)

- Inability to metabolize phenylalanine
- Dietary management:
  - Reduced Phe intake
  - Low Phe/Phe-free medical foods

Rocha, MacDonald. Pediatric Health Med Ther. 2016;7:155-63.

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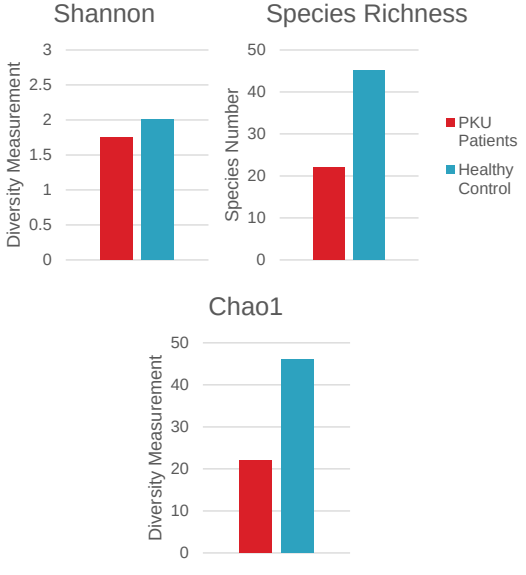
PKU and the Microbiome (Pinheiro de Oliveira, 2016)

- Reduction in diversity and richness
- Structurally distinct
  - ↑ Bacteroidetes, Verrucomicrobia, ***Akkermansia***, *Lachnospiraceae*, *Peptostreptococcaceae*, *Prevotella*
  - ↓ ***Alistipes***, *Parabacteroidetes*, *Family XIII UCG-001*



Pinheiro de Oliveira, et al. PLoS One. 2016;11(6):e0157513.

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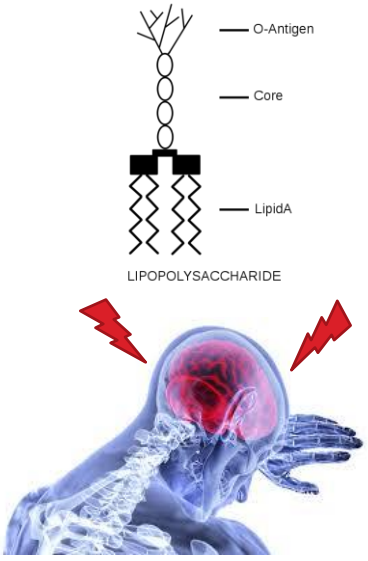
PKU and the Microbiome (Pinheiro de Oliveira, 2016)

- Functionally distinct microbiota
- Significant changes
  - ↑ **lipopolysaccharide biosynthesis proteins**, citrate cycle, …
  - ↓ **Glycolysis/Gluconeogenesis, starch and sucrose metabolism,** Phe, Tyr, Trp, Val, Leu, and Ile biosynthesis, …



Pinheiro de Oliveira, et al. PLoS One. 2016;11(6):e0157513.

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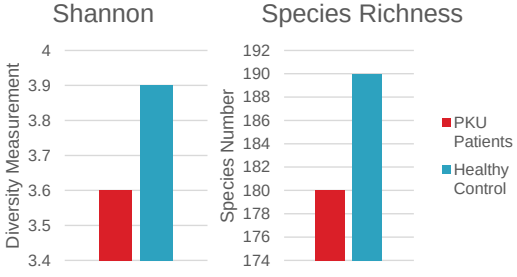
PKU and the Adult Microbiome (Mancilla, 2021)

- Trend in reduction of diversity and richness
- Structurally distinct
  - ↑ *Bifidobacterium*, *Bacillus*, **Alistipes**, *Clostridium*, ***Akkermansia***, *Bacteroides*
  - ↓ ***Faecalibacterium***, *Lactobacillus*, *Prophymonas*, *Blautia*, *Frisingicoccus*



Mancilla, et al. Microorganisms. 2021;9:530.

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PKU and the Adult Microbiome (Mancilla, 2021)

- Functionally distinct microbiota
- Significant changes
  - ↑ Biotin biosynthesis II, Superpathway of N-acetylneuraminate degradation, Allantoin degradation to glyoxylate III, Gluconeogenesis
  - ↓ Creatine degradation II, Superpathway of 2,3-butanediol biosynthesis

Mancilla, et al. Microorganisms. 2021;9:530.

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PKU Diet and the Microbiome (Bassanini, 2019)

- Focus on the effect of the PKU diet
- No changes in diversity detected
- Structurally distinct
  - ↑ *Lachnospiraceae*, *Blautia*, *Clostridium*
  - ↓ *Ruminococcacceae*, ***Faecalibacterium***, *Dialister*

Bassanini, et al. Front Cell Infect Microbiol. 2019;9:101.

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PKU Diet and the Microbiome (Verduci, 2018)

- Focus on SCFAs and butyrate production
- No assessment of communal diversity or structure
- Looked at shifts in known butyrate-producing taxa
  - ↓ ***Faecalibacterium*** *prausnitzii, Roseburia* spp.

Verduci, et al. Nutr Metab Cardiovasc Dis. 2018;28(4):385-92.

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Glycogen Storage Diseases (GSD)

- Inability to metabolize glycogen (synthesis, metabolism)
- Dietary therapy
  - **Uncooked cornstarch**
  - Polyunsaturated fats to reduce hyperlipidemia

1. Heller, et al. J Pediatr Gastroenterol Nutr. 2008;47 Suppl 1:S15-21. **2.** Bali, et al. Glycogen Storage Disease Type I. GeneReviews® [Internet]. 2006.

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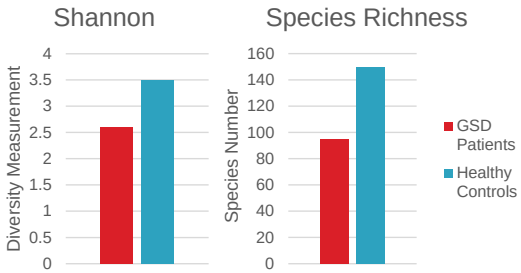
GSD and the Microbiome (Colonetti, 2019)

- Reduced species richness and Shannon diversity
- Structurally distinct
  - Presence/absence significant
  - ↑ Actinobacteria, Proteobacteria, *Escherchia/Shigella, Lactobacillus*
  - ↓ Euryarchaeota, *Coprococcus*, *Blautia*, *Anaerostipes*, *Odoribacter*, ***Faecalibacterium***



** All GSD patients in study were on uncooked cornstarch

Colonetti, et al. PLoS One. 2019;14(4):e0214582.

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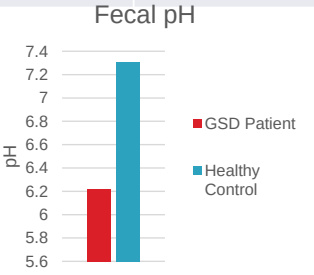
GSD and the Microbiome (Colonetti, 2019)

- Trends mirror inflammatory bowel disease (IBD) and cirrhosis
  - Diet-induced dysbiosis is clear
  - Does GSD alone induce dysbiosis??
- GSD patients on uncooked cornstarch had:
  - ↓ fecal pH
  - ↑ fecal carbohydrates

GSD	NAFLD Overlap
↑ Actino/Proteobacteria, *Escherchia/Shigella, Lactobacillus*	↑ *Lactobacillus*
↓ Euryarchaeota, *Coprococcus, Blautia, Anaerostipes, Odoribacter, Faecalibacterium*	↓ *Coprococcus, Blautia, Anaerostipes, Faecalibacterium*



1. Colonetti, et al. PLoS One. 2019;14(4):e0214582. **2.** Da Silva, et al. Sci Rep. 2018;8:144.

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Diet… What is the Effect in General?

- Diet plays a huge role in shaping gut microbiota naturally
- What impact does specialized diet play in the previous data?
- How do we study the disease effect independent of diet?
  - It is important to understand both simultaneously

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The Gut-Brain Axis and the Impact of Dysbiosis on IEM Disease Presentation

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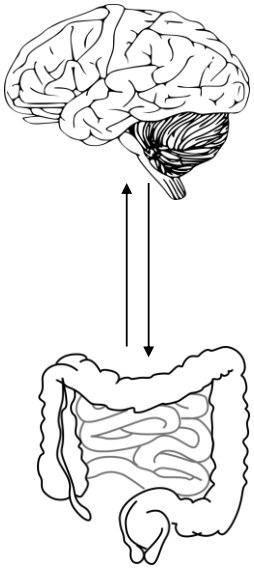
The Gut-Brain Axis

- A bidirectional communication pathway between the gut and the CNS
  - Traditionally viewed in terms of neurotransmitters
  - Uses other traditional physiological systems to operate
    - Endocrine system
    - Immune system



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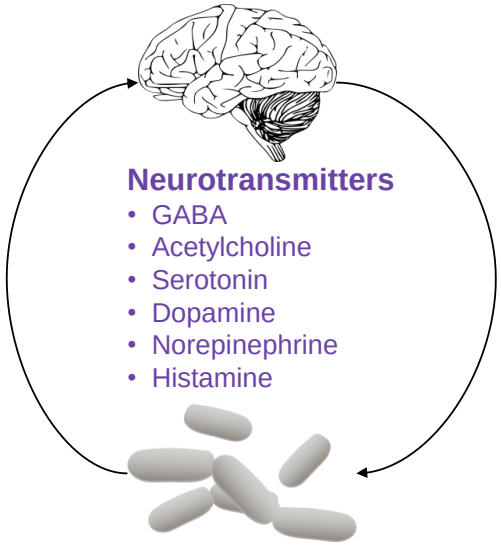
Host-Microbe Neurotransmitter Interactions

Producing bacteria
- Dopamine
  - *Bacillus subtilis*
  - *Staphylococcus aureus*
- Serotonin
  - *Klebsiella pneumoniae*
  - *Lactobacillus plantarum*
- Acetylcholine
  - *Lactobacillus plantarum*
- GABA
  - *Lactobacillus*
  - *Bifidobacterium*
  - *Escherichia coli*



Consuming bacteria
- Dopamine
  - *Escherichia coli* O157:H7 (EHEC)
  - *Pseudomonas aeruginosa*
- Serotonin
  - *Klebsiella pneumoniae*
  - *Lactobacillus plantarum*
- GABA
  - *Escherichia coli*

Strandwitz. Brain Res. 2018;1693:128-33.

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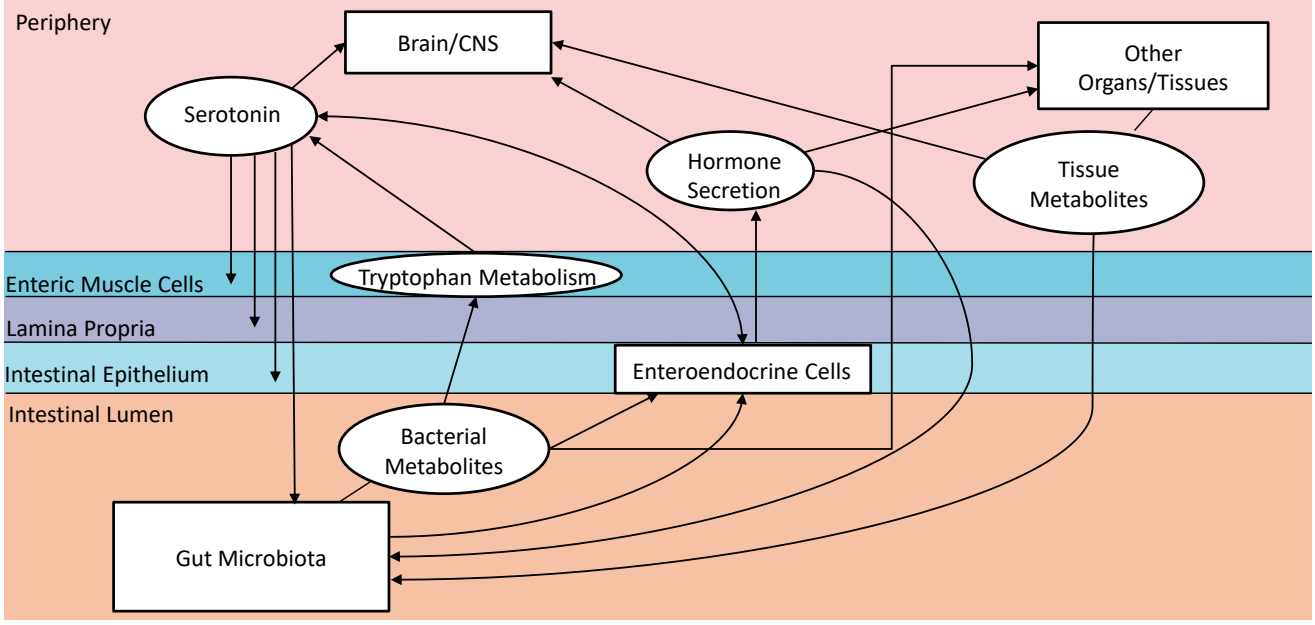
Host-Microbe Endocrine Interactions



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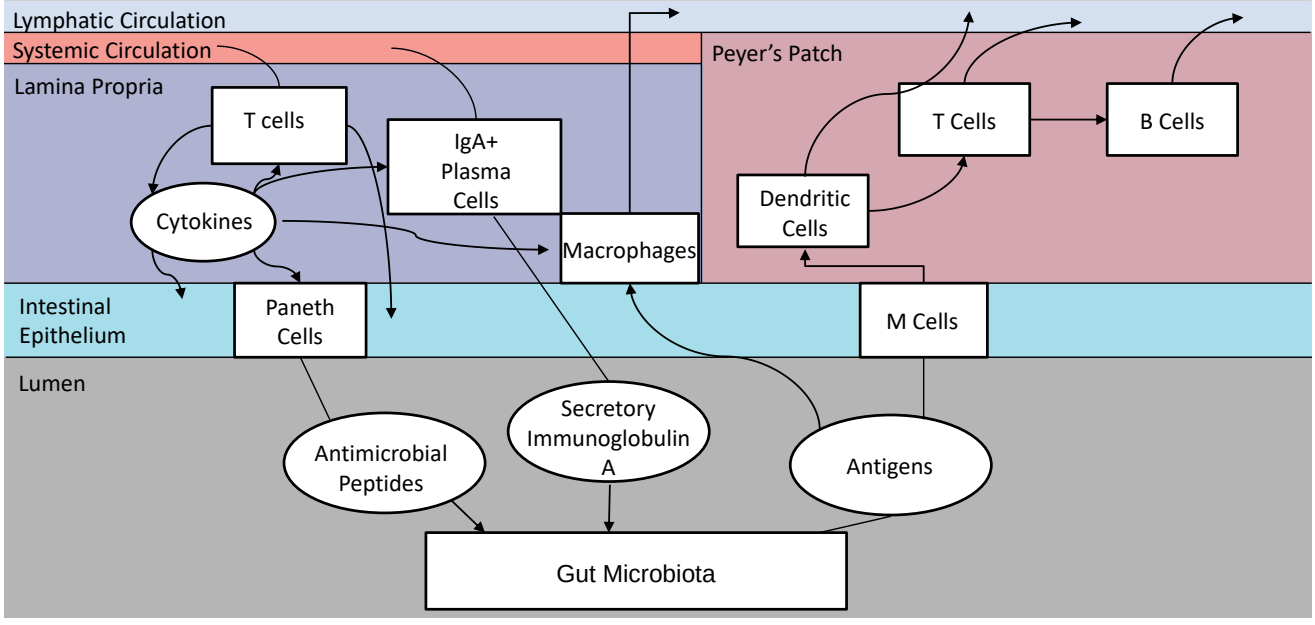
Host-Microbe Immune Interactions



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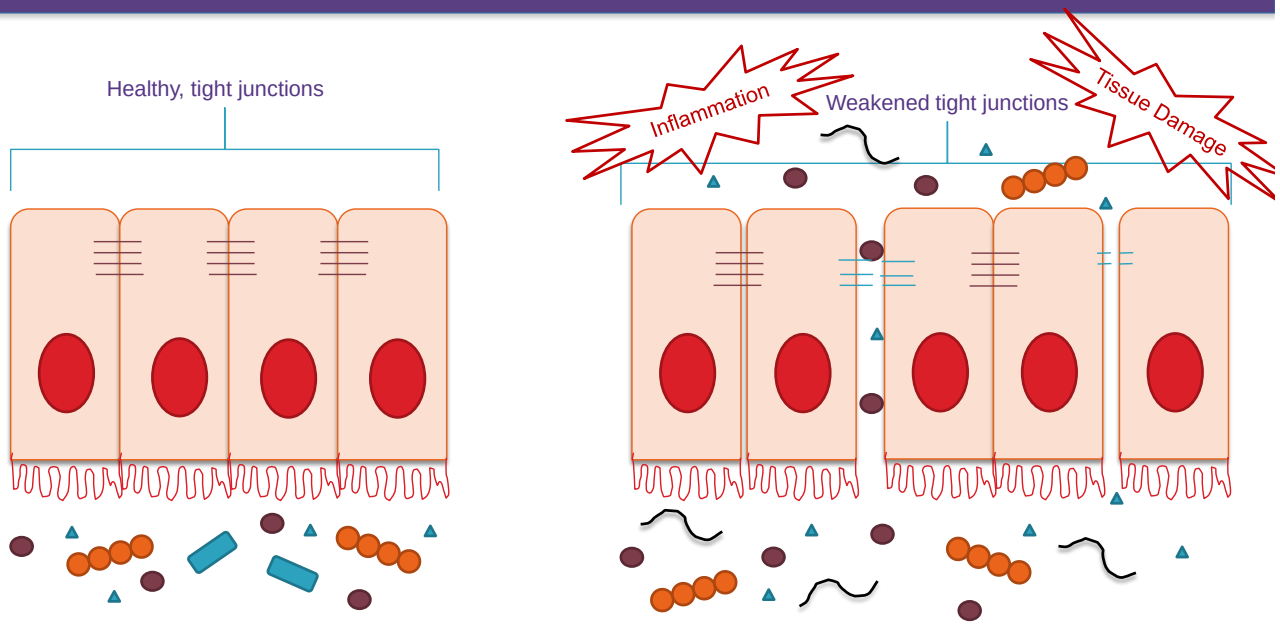
Dysbiosis and Leaky Gut



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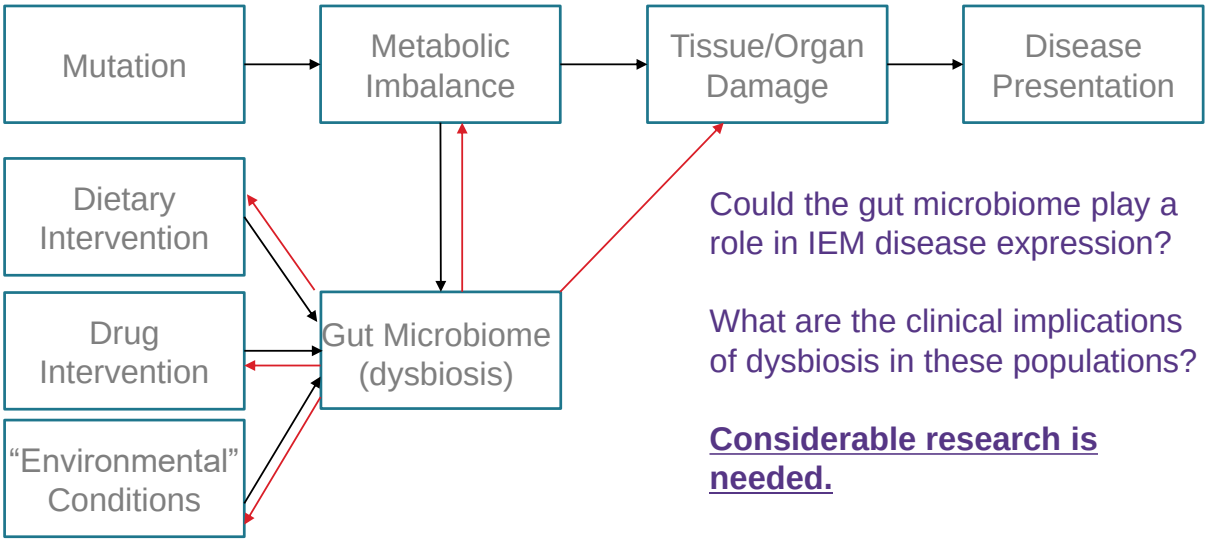
Impact of Dysbiosis on IEM Clinical Presentation



Could the gut microbiome play a role in IEM disease expression?

What are the clinical implications of dysbiosis in these populations?

Considerable research is needed.

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Pre-, Pro-, and Synbiotics in IEMs

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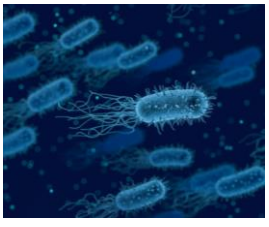
What are Pre-, Pro-, and Synbiotics?

Prebiotics

Substrates that are selectively utilized by host microorganisms, conferring a health benefit[1]

- Primarily oligosaccharide carbohydrates
  - Asparagus, garlic, onion, wheat, banana, rye, peas, …

Probiotics



Live microorganisms which when administered in adequate amounts confer a health benefit on the host[2]

- Which one depends on benefit of interest
  - *Lactobacillus rhamnosus GG* for diarrhea
  - *Saccharomyces boulardii* for adjunct treatment of *Helicobacter pylori*

Synbiotics

- Combination of pre-/probiotics together
  - Enhances probiotic and resident microbiota[3]
- Can be targeted:
  - Loaded with fructans plus lactic acid bacteria
  - Loaded with glucose derived oligosaccharides plus *Ruminococcus*
- Can decrease undesirable metabolite concentrations

1. Gibson, et al. Nat Rev Gastroenterol Hepatol. 2017;14(8):491-502. **2.** Hill, et al. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-14. **3.** Bengmark. Gastroenterol Clin North Am. 2005;34(3):413-36.

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Do They Work for Disease - IBD?

	Efficacious	Non-Efficacious	Citations
Prebiotics	Fructans	–	Marushko, et al. Modern Pediatrics. 2013:66-72.
Probiotics	*Lactobacillus spp.*, *Enterococcus faecium*, *Saccharomyces boulardii*	*Lactobacillus acidophilus La-5* and *Bifidobacterium animalis* subsp. lactis BB-12	Tomasello, et al. J Biol Regul Homeost Agents, 2015;29(2):265-72. Wildt, et al. J Crohns Colitis. 2011;5(2):115-21.
Synbiotics	*Bifidobacterium longum* and Synergy 1 (Raftilose®)	–	Steed, et al. Aliment Pharmacol Ther. 2010;32(7):872-83.

- Recall… IBD is common in GSD

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Do They Work for IEMs?

- Not much data for IEMs…

IEM	Type	Efficacy?	Citations
PKU	Probiotic - pHENOMMenal	Yes in mouse model	Durrer, et al. PLoS One. 2017;12(5):e0176286.
Trimethylaminuria	Probiotic - *Methanomassiliicoccus luminyensis B10*	Theoretical prediction	Brugère, et al. Gut Microbes. 2014;5(1):5-10.
Hyperoxaluria	Probiotics – *Bifidobacterium animalis* subsp. lactis	Yes in mouse model	Klimesova, et al. Urolithiasis. 2015;43(2):107-17.
	Oxalobacter formigenes		Hatch, et al. Am J Physiol Gastrointest Liver Physiol. 2011;300(3):G461-9.
	Synbiotics - Oxadrop® and Agri-King Synbiotic	No in patients	Lieske, et al. Kidney Int. 2010;78(11):1178-85.

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Theoretically, What Can We Do About Dysbiosis?

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Surveillance

Monitoring patient microbiota

Dietary Supplementation

Leveraging diet strategically

Experimental Technologies

Continued research into novel technologies

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Microbiome Surveillance

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- What if we tracked a patient's gut microbiome as disease progresses?
  - I do this for Succinic Semialdehyde Dehydrogenase Deficiency
    - IEM of GABA metabolism
- Far from being a clinical service right now…

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Dietary Supplementation

- Preemptive strategies
  - Encourage breastfeeding if possible
  - Diversify patient's diet within dietary restrictions
    - Particularly foods containing fiber
  - Choose formulas and medical foods with prebiotics
- Proactive strategies
  - Directed pre/pro/synbiotics targeting "reduced" taxa
  - Recommending lifestyle changes to promote healthy microbiota

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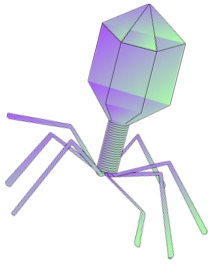
Experimental Technologies

- Bacteriophage Technology
  - Viruses that attack certain bacteria only
  - Creating smart "phage cocktails" could allow for tailored changes to the microbiome structure
- Fecal Transplantation
  - Using stool from a healthy donor to reseed a healthy gut community
  - Last ditch effort to start the gut microbiome
- Transgenic probiotics
  - Leverage microbial metabolism strategically



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Conclusions

1. Gut microbiome is essential for healthy physiology.
2. IEMs may be associated with dysbiosis.
3. Dysbiosis may be a pathogenic factor in IEMs that should be considered.
4. Modulating the gut microbiome may be a tool to mitigate IEM severity.

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MAYO CLINIC
CLIR - Collaborative Laboratory Integrated Reports

ssadh
Succinic Semialdehyde Dehydrogenase Deficiency Association

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Thank you!

To obtain a certificate of attendance for 1 CE hour, please complete a brief survey which you can access via the QR code below or the following link: https://www.surveymonkey.com/r/Dysbiosis_IEM

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