A New Determinant of Health

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OBJECTIVES
1. define the human microbiome
2. new ways of identifying microbial diversity
3. gut microbiome function
4. implications of the microbiome on research, physiology and treatment

PRESENTATION IS DIVIDED INTO SEGMENTS
- What is it?
- Where does it come from?
- Why didn’t we know about this before?
- What does it do?
- Why should we care? (i.e. put it in clinical context...)
- Questions anyone?
- Resources
What is it?

THE HUMAN MICROBIOME

What is it?
THE MICROBIOME - WHAT IS IT?

- **Microbes:** not a scientific term.....lots of little things...

- **Bacteria and Archaea:** both single celled, no nucleus, same shapes (bacilli, cocci, spirochetes – flagellated, with cilia etc but differ at the RNA level, and so have different functions

- Archaea were long not recognized to be different from bacteria because we classified by shape and not genes! (1977 - Carl Woese – Discovered the third domain of life...)

- Archaea tend to live in extreme environments (salt flats, hot springs, glaciers – we as humans are closer to archaea than bacteria... mostly found in soil and sea water

- **Eukaryotes:** usually multi-celled, nucleus which contains DNA

- **Viruses** – technically not alive (not on tree of life)
- **Bacteriophages**
- **Fungi**
THE TREE OF LIFE

Microbes are the most numerous organisms on earth. 10 orders of magnitude more microbial cells on earth than stars in the universe!
THE TREE OF LIFE

Phylogenetic Tree of Life

Bacteria
- Spirochetes
- Proteobacteria
- Cyanobacteria
- Planctomyces
- Bacteroides
- Cytophaga
- Thermotoga
- Aquifex

Archaea
- Green Filamentous bacteria
- Methanosarcina
- Methanobacterium
- Methanococcus
- T. celer
- Thermoproteus
- Pyrodictium

Eukaryota
- Entamoebae
- Slime molds
- Animals
- Fungi
- Plants
- Ciliates
- Flagellates
- Trichomonads
- Microsporidia
- Diplomonads
Definition:

The collection of microbes on and in our body

When we refer to the collective genes of these microbes we talk about the MICROBIOME

They include bacteria, archaea, viruses and micro-eukaryotes and fungi. But due to technical limitations, research focuses on BACTERIA mostly.

When referring to them as organisms, we talk about MICROBIOTA (i.e. formerly known as “flora”)

Let's put this in the HUMAN context...
WHO'S AFRAID OF A FEW LITTLE MICROBIES?

**CELLS**
- 10 trillion human cells vs 100 trillion microbial cells
- At a CELLULAR level we’re 10% human
- About as much as your left foot!

**GENES**
- 20K human genes.
- We harbor approx. 20 million microbial genes.
- So, in terms of our GENETIC make up, our bodies are in fact less than 1% human...
WHO'S AFRAID OF A FEW LITTLE MICROBES?

SIMILARITIES

- The chimpanzee genome was sequenced for the first time in 2005. It was found to differ from the human genome (nucleotide by nucleotide) by 1.23%
- From human to human we share 99.9% of DNA

DIFFERENCES

- But in terms of our microbiome, we are 90% different from one another, sharing only 10% of our microbiome features
- Is what makes us unique our microbiome?
- Same bacteria everywhere?
- In distinct habitats, distinct ecosystems
  - Costello et al, 2009
- In each habitat, these microbes have specific functions and produce proteins and metabolites that directly interact with the human host
- Proteomics and Metabolomics, Metagenomics...
IN THE HUMAN MICROBIOME, DIVERSITY IS KING

- **Skin**
  - Distinct ecosystems
    - From Costello et al Human ecology 2009
  - Arm pit and forehead: least diversity, high numbers
  - Palm for the hand - highest diversity. Left and Right hand have consistently different biomes
  - More diversity, less mosquito bites!
  - Metagenomics

- **Respiratory tract**
  - Increased nares bacterial diversity, associated with less asthma and allergies
  - MRSA: is “contained” by a greater diversity of innocuous bacteria

- **Oral mucosa**
  - Not all bacteria are bad, most in fact, protect us from pathogenic bacterial foothold
FOR THE HUMAN MICROBIOME, DIVERSITY IS KING

- **GUT**
  - Epicenter of microbial activity
  - 95% of our microbiome resides here
  - Distinct populations:
  - Large and small intestines have very different microbial profile
  - Poop is an excellent representation of the bacterial populations of the large intestine
  - MORE TO COME...

- **Breast Milk**
  - Long thought to be sterile, serves as a delivery vehicle of bifido bacteria to the gut
  - 1/3 of nutrients are oligosaccharides that exclusively feed bifido and lacto bacilli – were absent in formulas/ cows milk
  - Breast milk increases bifido bacteria by 300% w/in the first 4 days of life, suppressing pathogenic bacteria
    - [Vandenplast Y Br J. Nutr 2002](#)

- **Placenta**
  - Now we know this organ not to be sterile. In fact, it harbors the DNA fingerprints of urinary tract infections and periodontal infections

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[1](#)
WHERE DOES IT COME FROM?

http://learn.genetics.utah.edu/content/microbiome/changing/
HUMAN MICROBIOME ORIGINS

- Affected by maternal health (placental colonization)
- mode of delivery
- breast feeding
- skin to skin contact
- natural exposures to the environment including (geophagia) *

DIET
- Antibiotics
- Pesticides?
- GMO crops?
- Microbiome is established by age 3

* “Old Friends” Theory

Laura Wolfer MA (Cultivate Your Nature)
Prof. Maria Gloria Dominguez Bello at NYU

Soring rates of C-sections in Latin America (Brazil: private hospitals 80%/ 50% state)

Infants born via C-section have higher rates of asthma, obesity, type 1 diabetes, IBD

Prospective study of infants born via c-section to assess effects of maternal vaginal microbiome “implant”

Also, dearth / absence of Bacteroides bacteria

A word on antibiotics....

Sweden uses 40% of the antibiotics used in the US, not counting the antibiotics used in agriculture

Single course can cause microbial shifts that can take up to a year in some cases to recover but a second course within this time frame will change the microbiome. Dethlefsen et al 2010 PNAS

Antibiotic exposure increases the risk of obesity
  - L Trasande at al 2012 International Journal of Obesity
WHY DIDN'T WE KNOW ABOUT THIS BEFORE?

THE MICROBIOME

WHY DIDN'T WE KNOW ABOUT THIS BEFORE?
FROM ANIMACULES TO MOLECULES...
A LITTLE HISTORY

Microbiology – a visualization technique:

Microscopy (Leeuwenhoek- late 1600’s),
- Later, staining techniques further helped w/ identification, still not truly able to differentiate one cocci from another

Culture dependent techniques –
- Developed in 1880 by Koch (Koch's postulate) used culture media to detect bacteria.
- Visualizing ONLY those microbes that grew on culture media:
- Think ecoli!
  - Most gut microbes CAN NOT BE CULTURED, E coli however, grows readily in culture
  - Its NOT the most important or the most abundant gut bacteria! (Bacteroides and Firmicutes are)

QUIZ: What % of microbes can be cultured? ....
FROM ANIMACULES TO MOLECULES...A LITTLE HISTORY

- Microbiology – a visualization technique:

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- Culture dependent techniques –
  - Developed in 1880 by Koch (Koch's postulate) used culture media to detect bacteria.
  - Visualizing ONLY microbes that grew on culture media: caveat #1
    - Think ecoli!
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- QUIZ: what % of microbes can be cultured? .... Only 0.1 to 1%
FROM ANIMACULES TO MOLECULES...A LITTLE HISTORY

- 1953: double DNA helix is established
- 1970: DNA sequencing was born – painfully slowly
- 1987: first “automated” DNA sequencing machine
- 1990: NIH starts to sequence DNA
- 1995: The Institute for Genomic Research publishes the first complete genome: Haemophilus Influenzae
- 2003: National Human Genome Research Institute announced the successful completion of the Human Genome Project
- Between 2001-2007 16s rRNA analysis the most pivotal microbiological tool:
  - By 2008: 215 novel bacterial species, 29 of which belong to novel genera, have been discovered from human specimens alone
  - 1980: 12 bacterial phyla (cultured);
  - 2014: 100+ phyla (sequenced)
16S RNA SEQUENCING

Microbiome sample → Nucleic acid extraction/purification → 16S rRNA sequencing:
- PCR amplify 16S rRNA gene
- Sequence
- Group sequences into OTUs
- Compare OTU sequences to databases
- Identification of:
  - Species
  - Relative abundance of species within sample

Total microbiome DNA sequencing:
- Next-generation sequencing of total DNA from the microbiome sample
- Filter host DNA sequences
- Compare microbial sequences to databases and reference genomes
- Identification of:
  - Species
  - Relative abundance of species within sample
  - Genes
- Identification of:
  - Variants
  - Polymorphisms
  - Functional information
16s rRNA: bacteria and archaea only
- It has 15K nucleotides
- Preserved throughout species
- But has a small region (V4) that is variable – driven by evolutionary adaptations – and this is each species “finger print” - where the magic happens....
16S RNA SEQUENCING

- **SHOT GUN!**
- Extract all the DNA
- Filter the host DNA (nuisance human/ plant/ fungal etc)
- Compare to database
- Presto: not only positive ID and diversity (alpha and beta) but bacterial functions: proteins*, transcription, metabolites
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These bacterial proteins interact with the host; so far, over 1000 such proteins have been identified
THE MICROBIOME

WHAT DOES IT DO?
RECAP...

- We know what it is...
- We know where it comes from...sort of...
- We know how it’s measured/identified...
- We understand some of the factors that can modify it...
  - Age (diversity/stability)
  - Diet! (fat/ meats vs fiber, firmicutes vs bacteroides)
  - Environment, environmental exposures
  - Genes
    - (chicken or the egg - Crohns and bacterial diversity or epigenetic effects, twin studies show greater environmental dependence..)
  - Antibiotics! (to bounce back or not to bounce back – that is the question... Dethlefsen et al 2010 PNAS)
  - Others still to be determined
Delivers **nutrients** by breaking down food products in our gut

- Epigenetic effects (modifies **gene expression** - inflammation)
- Molecules with **hormonal effects** (brain, metabolism and immune system) i.e. affecting Leptin and mimicking neurotransmitters
- **Modulates the immune system**
  - "Old Friends Theory" – dialing down immune response vs outcompeting pathogenic bacteria
Defense against colonization by nonnative/opportunistic pathogens - think fecal transplants as potential first line therapy for chronic C-diff infection

- 3 x increase in 1 decade - now 250,000 hospitalizations and 14,000 deaths per year. More than even MRSA infections.

- Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile
  93% vs 31% cure rate: transplant vs vancomycin

Co-evolution: H Pylori, Oxylobacter, consider microbial extinctions...
Dr. Martin Blaser has studied the role of bacteria in human disease for over 30 years. He is the director of the Human Microbiome Program at NYU.
“Missing Microbes” 2014 (a MUST READ)
Gut Microbiota and Clinical Disease: Obesity and Nonalcoholic Fatty Liver Disease

Ji Sook Park, Ji Hyun Seo, and Hee-Shang Youn
First, the obese gut microbiota increases both the capacity to harvest energy from the diet and the accumulation of fat in adipose tissue and the liver by altering host metabolism. Studies in germ-free and conventionalized mice revealed that the microbiota promoted absorption of monosaccharides from the gut lumen and adipocyte hypertrophy by suppressing fasting-induced adipocyte factor (Fiaf) in the intestine, and suggest that the gut microbiota is an important environmental factor that affects energy harvest from the diet and energy storage in the host [13,27]. Germ-free mice were protected from diet-induced obesity by increased AMP-activated protein kinase (AMPK) activity. In conventionalized mice fed a high-fat, high sugar western diet, the gut microbiota suppressed AMPK activity in the gastrocnemius muscle and the liver [28]. Turnbaugh et al. [29] reported that the obese microbiome (i.e., the combined obese microbiota, their genetic elements and environmental interactions) has an increased capacity for dietary energy harvest because it is enriched in environmental gene tags encoding many enzymes involved in breaking down usually indigestible dietary polysaccharides. Second, the gut microbiota regulates entero-endocrine cells, promoting the release of several gut hormones [30-33]. Gut microbiota-mediated increase in adiposity was blunted in conventionally raised G protein-coupled receptor 41 (Gpr41) deficient mice [30]. Gpr41 is a receptor for the binding of short chain fatty acids produced by fermentation of polysaccharides by gut microbes. According to Samuel et al. [30], expression of peptide YY (PYY) was lower in both Gpr41-deficient germ-free and conventionalized mice than in Gpr41 intact mice. Reduced expression of PYY resulted in increased intestinal transit time and reduced dietary energy harvest. Dietary fructo-oligo-saccharides increased the proportion of bifidobacteria in the distal intestine, leading to increased colonic fermentation and glucagon-like peptide 1 (GLP1) levels, and decreased food intake, fat mass, and hepatic steatosis [31-33]. Third, obese gut microbiota seem to provoke chronic low-grade inflammation in the host gut [34-36]. Chronic experimental metabolic endotoxemia-induced obesity, diabetes, and liver insulin resistance triggered the expression of inflammatory factors similar to those associated with a high-fat diet by a CD14-dependent mechanism [34]. According to de La Serre et al. [35], obesity in rats induced by a high-fat diet changed the gut microbiota and increased toll-like receptor 4 (TLR4) activation. They hypothesized that the activated TLR4 provoked gastrointestinal inflammation that was associated with the appearance of hyperphagia and an obese phenotype.
Colorectal cancers:
- Associated with increased numbers of *fusobacterium nucleatum* around the tumor sites, known to be inflammatory
- Cause or effect?

Chronic disease
- Associated with decreased overall gut diversity
  - IBD – Crohns and UC
    - Vastly different microbiome
    - Much more susceptible to diversity changes with antibiotic administration
Why should we care?

THE MICROBIOME

Why should we care?
Why should we care

- It’s so much more than just about OTC supplements
- Pharmaceutical gold rush in the works, biotech start ups, patenting of bacteria, ethics of fecal transplants for “behavior modification” or “weight loss”...
- We need to discern correlation vs. causation
- We need to counsel patients on the effects of diet, lifestyle and ANTIBIOTICS on the microbiome
- New variable in research studies
- It’s a new organ....
THE HUMAN MICROBIOME - A NEW ORGAN

- 3 -5 lbs
- 95% found in the gut
- Has multiple complex functions including:
  - immune system modulation
  - Influences behavior
  - Intermediary in drug reactions (acetaminophen)
  - Nutrient delivery (food digestion)
  - Determines metabolism rate
- Lets not throw it out like the Egyptians...

Based on a lecture by Dr Robert Knight
IN SUMMARY

- A new organ – thanks to new DNA sequencing technologies
- New knowledge about the human microbiome has created a paradigm shift in our view of human biology and human disease
  - bacterial, viral and fungal species, do not cause disease but, in fact, are required for normal human development and for lifelong
- Needs to be considered a variable in research studies
- Research is rapidly developing on this front – stay tuned!
- Classic Pitfalls:
  - ascribing everything to the microbiome vs discerning compelling new information
  - correlation vs. causation; over simplifying things we don’t yet quite understand...
- The clinician needs to be able to talk about the microbiome as it becomes a mainstream concept (see NYT...)
  - http://www.nytimes.com/2014/11/02/opinion/sunday/there-is-no-healthy-microbiome.html?_r=0  Ed Young
THE MICROBIOME: RESOURCES

- American Gut Project: americangut.org
- Human Food Project: humanfoodproject.com
- Small Things Considered Blog: http://schaechter.asmblog.org/schaechter/
- Stories about health and the microbiome from NPR: www.npr.org/tags/172709084/microbiome
- The Invisible Universe of the Human Microbiome: https://www.youtube.com/watch?v=5DTtrENdWvvM
- Excellent trailer for an upcoming film series about the gut microbiome: redefininghuman.com
- Twitter
- Coursera
- Crowd funding
QUESTIONS?

Thank you!

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