

# The Role of the Gut Microbiota in Obesity in the Old Order Amish

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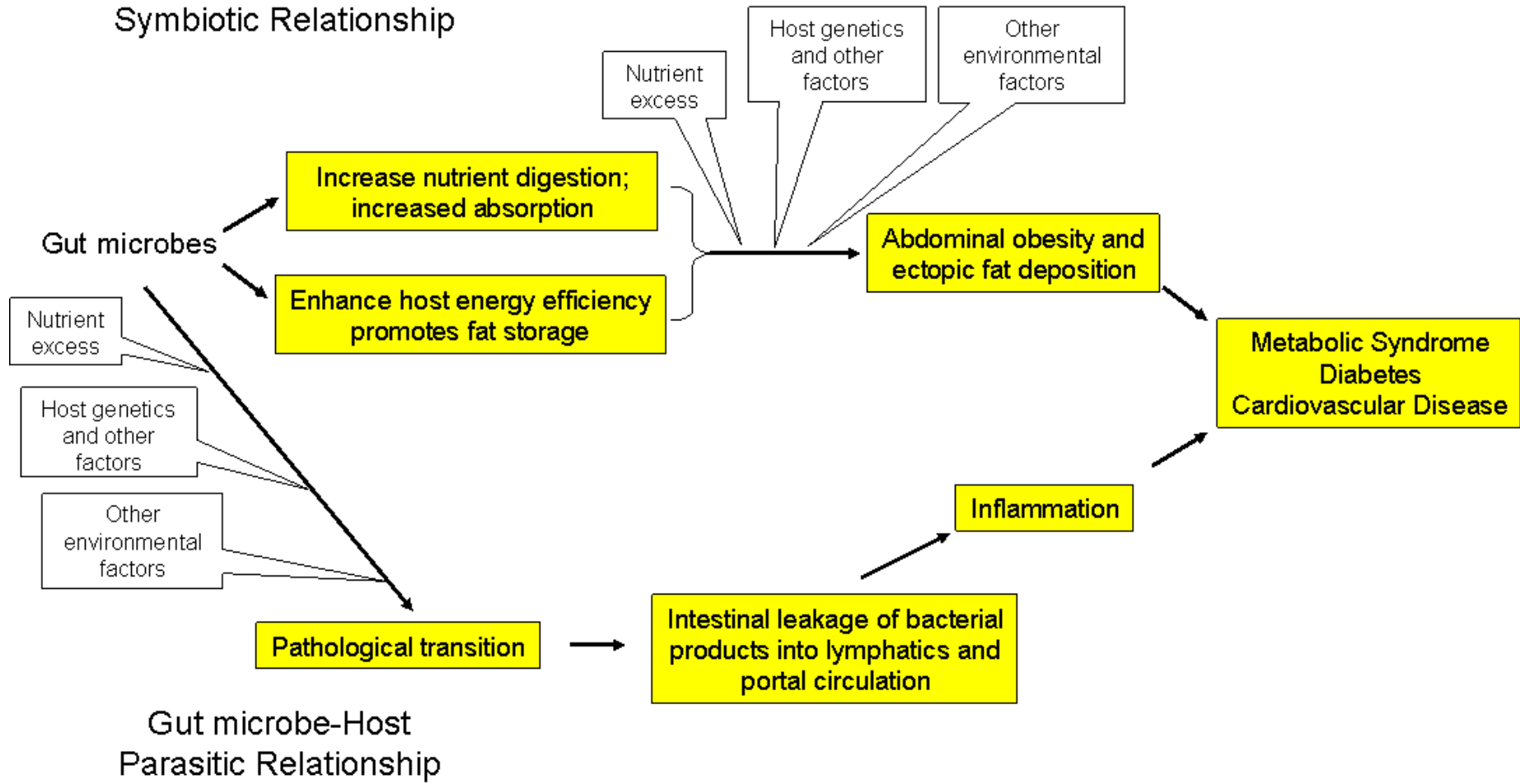
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**UH3 DK83982**

# Gut microbe-Host Symbiotic Relationship



# Evidence for a Role of the Gut Microbiome in Obesity – The facts in humans

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- Humans share a “core microbiome” but there is marked inter-individual variation in gut microbiome.
  - Functional state of community may be more similar among individuals than bacterial species composition
  - Less intra-individual variability over time
- Family members share more similar gut microbiotas than unrelated individuals (Turnbaugh et al 2009).
  - Dizygotic twin pairs have similar degree of variance as do dizygotic twin pairs (role of the environment)
- Continuing controversies:
  - Obese humans may have different gut microbiome content (lower B/F) than lean humans (e.g., Kalliomaki et al 2008 vs. Duncan et al 2008)
  - The gut microbiome changes with diet/weight loss (e.g, Nadal et al 2008 vs. Duncan et al 2008) and after gastric bypass surgery (Zhang et al 2009)



# Old Order Amish

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Approximately 200 Amish founders came from Europe to Lancaster County in the early 1700s

The Amish population in Lancaster County, PA has grown to ~30,000

Closed population with a fixed gene pool, large families, and genealogies dating back 14 generations

Amish Research Clinic has enrolled more than 4,000 subjects



Alan Shuldiner, MD

# Old Order Amish and Obesity

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- Extensive clinical metadata
- Similar, pre-World War II rural diet (neither low carbohydrate nor low fat)
- Limited food intake between meals
- Limited medications (antibiotics)
- Their occupations and range of physical activity have been well-characterized (Rampersaud et al., 2008, Archives Int. Med. 168; 1791-1797)
- GWAS on nearly 4,000 OAA are available
- Extent and distribution of linkage disequilibrium in the OAA is similar to that observed in the US Utah (CEU) population from the International HapMap Project (VanHout et al., 2009, Genet Epidemiol. Aug 2008)
- Interaction of obesity alleles and physical activity has been studied (Rampersaud et al., 2008, Archives Int. Med. 168; 1791-1797)

# Amish HMP – Study Design

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**Hypothesis:** The gut microbiota influences host energy homeostasis, metabolism, and inflammation, and is an important determinant of obesity and its adverse health consequences.

- **Phase I - Cross-sectional**
  - Compare gut microbiota in fecal samples from non-obese, overweight, obese with and without features of metabolic syndrome (16S rRNA)
  - Compare two samples in same subject 3-12 months apart



**Expected result: The microbiome will be similar in a given subject over time and will differ between lean and obese subjects and between obese subjects with metabolic syndrome and obese subjects without metabolic syndrome.**

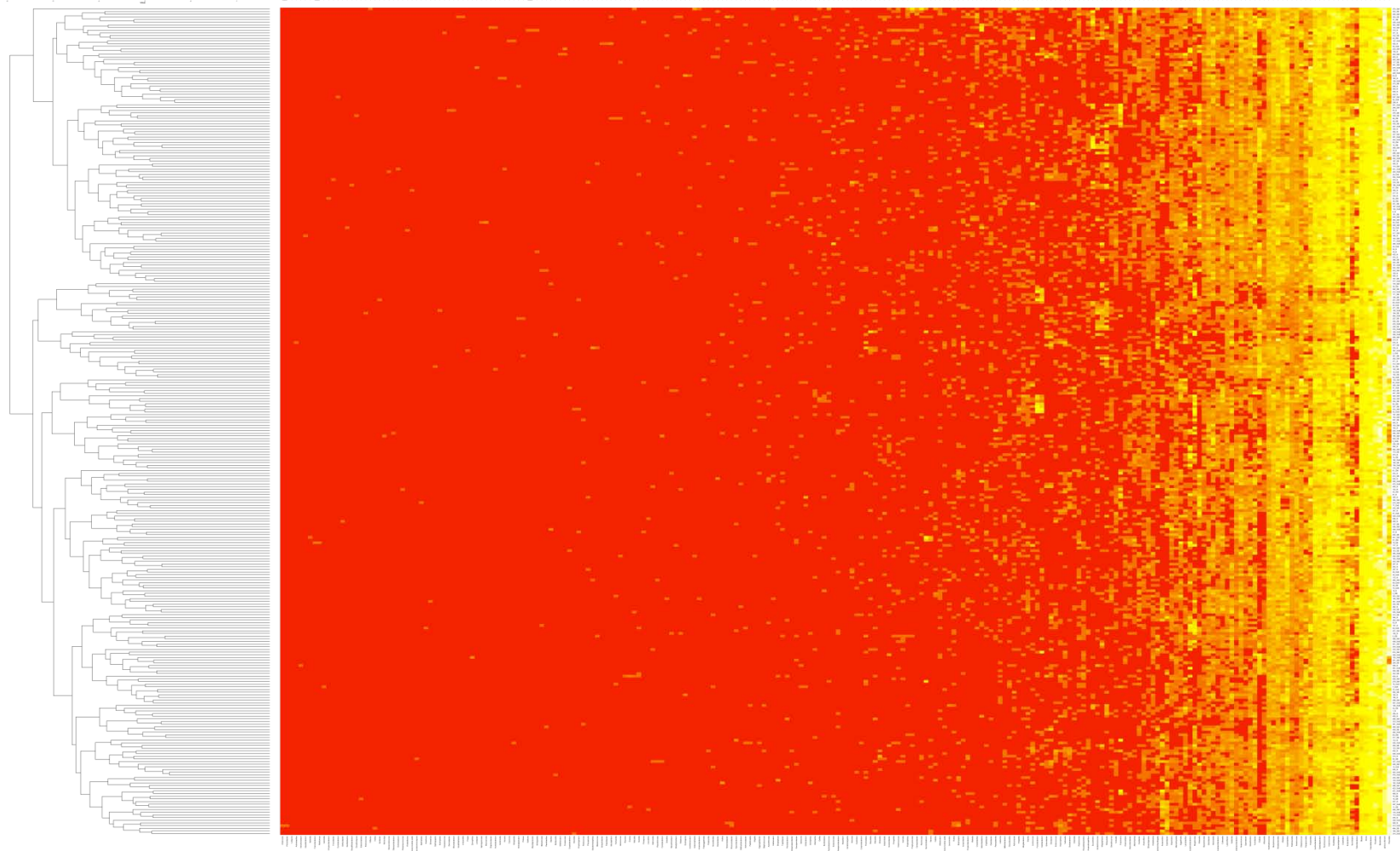
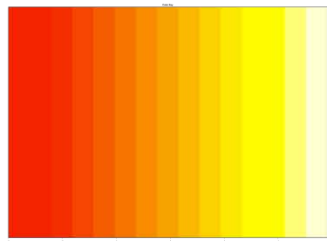
# Table 1. Characteristics of Subjects

	Normal (n=75)	Overweight benign (n=68)	Overweight malignant (n=22)	Obese benign (n=66)	Obese malignant (n=79)
<b>Age</b> (yr)	43.7 ± 15.1	43.0 ± 11.3	51.6.4 ± 10.1	48.1 ± 10.6	56.2 ± 11.6
<b>Gender</b> (% female)	54.7	48.5	36.4	77.3	82.3
<b>BMI</b> (kg/m <sup>2</sup> )	22.4 ± 1.8	26.9 ± 1.2	27.9 ± 1.2	33.3 ± 3.1	34.6 ± 4.1
<b>Waist Circ.</b> (cm)	78.3 ± 7.4	89.3 ± 8.6	97.3 ± 6.6	98.1 ± 9.0	101.4 ± 9.6
<b>Systolic BP</b> (mmHg)	111.8 ± 12.1	112.3 ± 8.8	129.0 ± 13.0	115.3 ± 9.5	129.7 ± 16.7
<b>Diastolic BP</b>	67.8 ± 7.1	67.5 ± 6.0	77.7 ± 7.9	70.1 ± 6.8	75.7 ± 10.3
<b>CHOL</b> (mg/dl)	204.4 ± 47.2	202.6 ± 40.2	216.3 ± 36.1	218.2 ± 53.9	219.5 ± 46.7
<b>HDL-C</b> (mg/dl)	67.8 ± 15.0	58.5 ± 12.0	52.7 ± 12.6	61.2 ± 11.8	52.3 ± 14.0
<b>LDL-C</b> (mg/dl)	126.4 ± 43.2	130.9 ± 36.0	141.0 ± 31.3	141.4 ± 50.0	141.9 ± 42.5
<b>TG</b> (mg/dl)	54.7 ± 21.7	66.0 ± 24.4	113.2 ± 87.4	78.2 ± 27.1	126.6 ± 60.7
<b>Glucose</b> (mg/dl)	82.2 ± 6.3	84.4 ± 6.1	89.0 ± 13.3	87.4 ± 6.7	94.0 ± 12.9

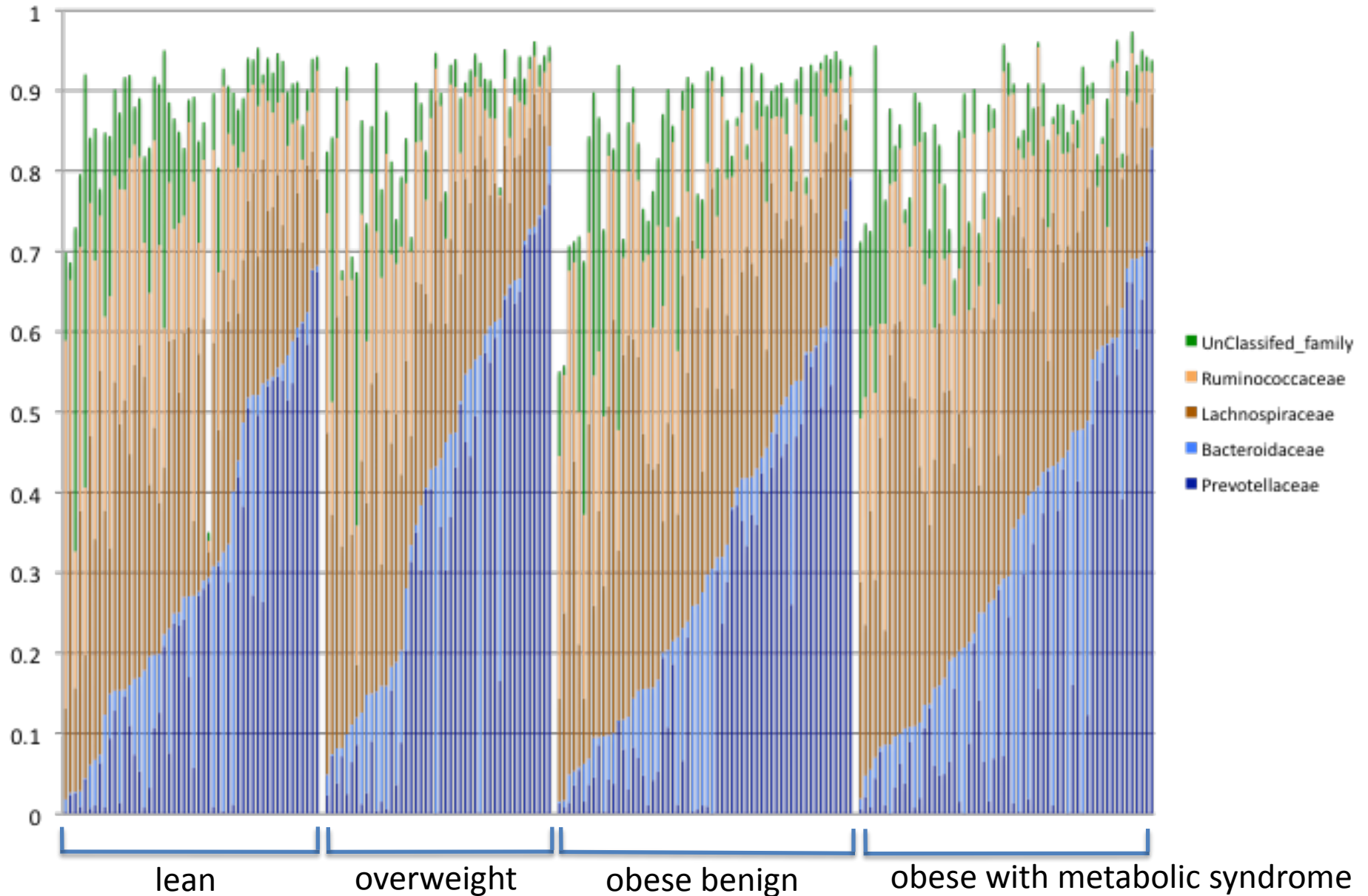
Obese benign: No features of metabolic syndrome (excluding waist circumference)

Obese malignant: Increased waist circumference plus least one additional feature of metabolic syndrome

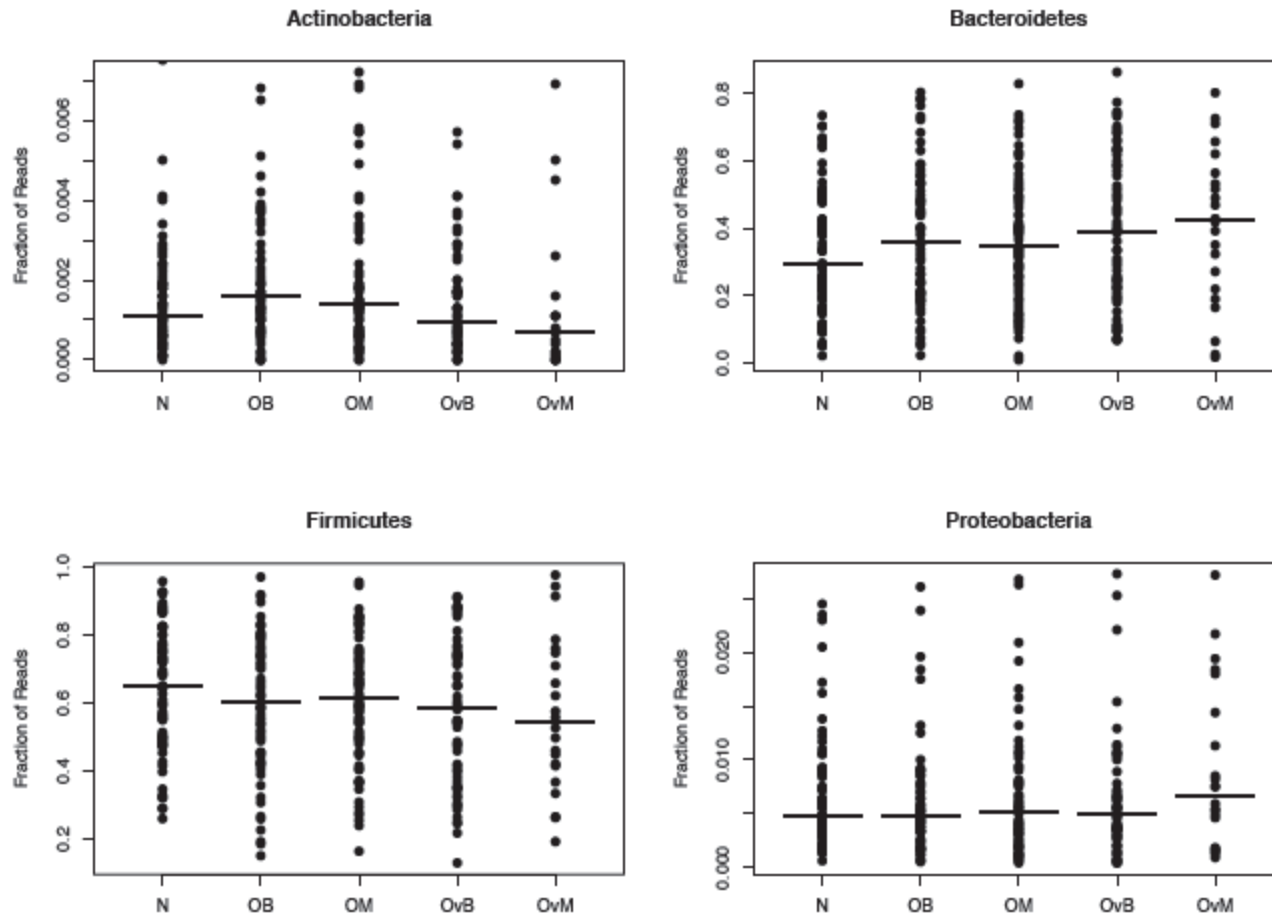
# Heat map of 16S rRNA data from cohort of OOA



# Family-level Phylogenetic Binning



# Relative Abundance by Phylum



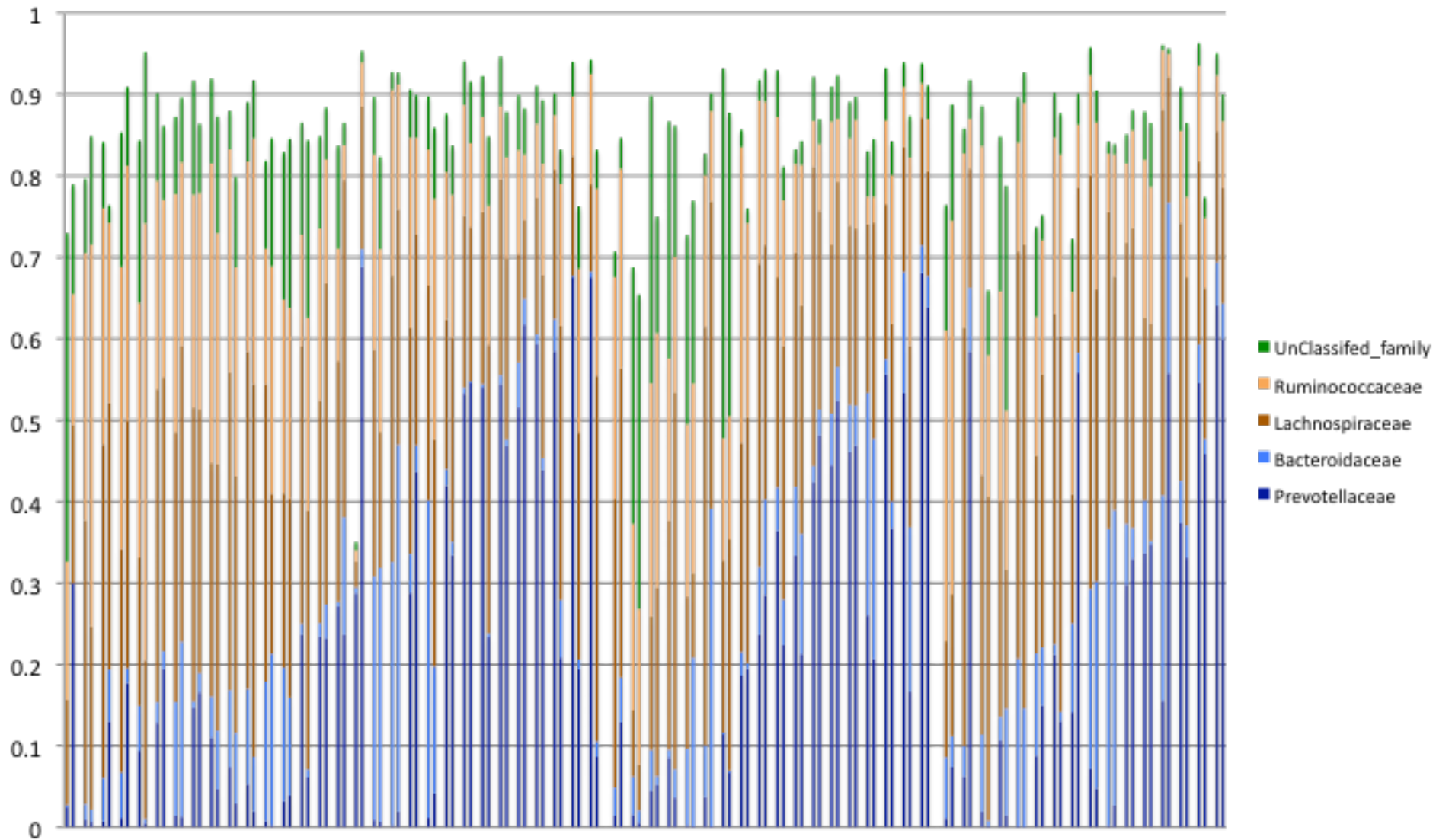
## Bacteroidetes: Firmicutes ratio

Lean: 0.47 (0.023 – 2.8)

Obese: 0.61 (0.015 – 5.37)

Overweight: 0.69 (0.047 – 5.9)

# Stability of Gut Microbiome Communities Over Time



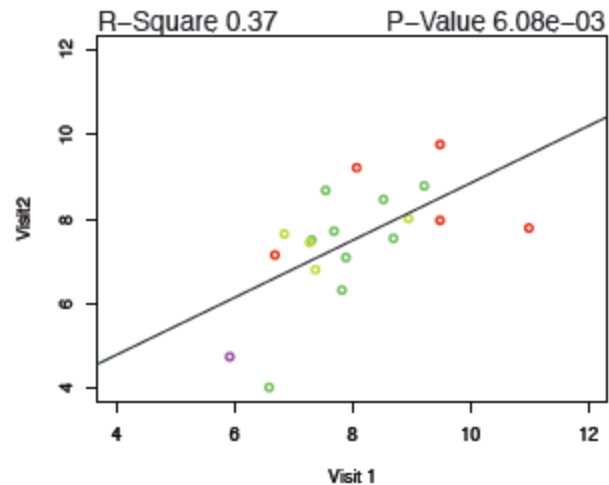
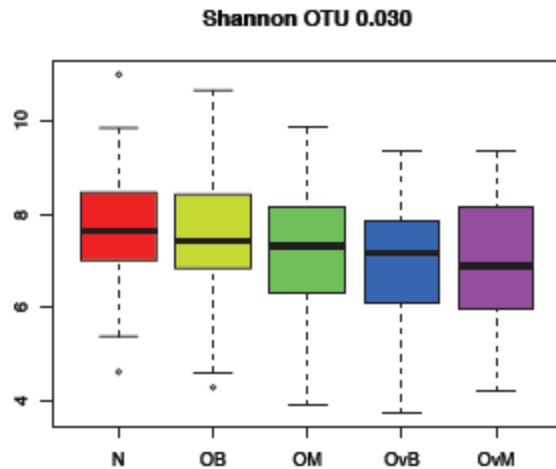
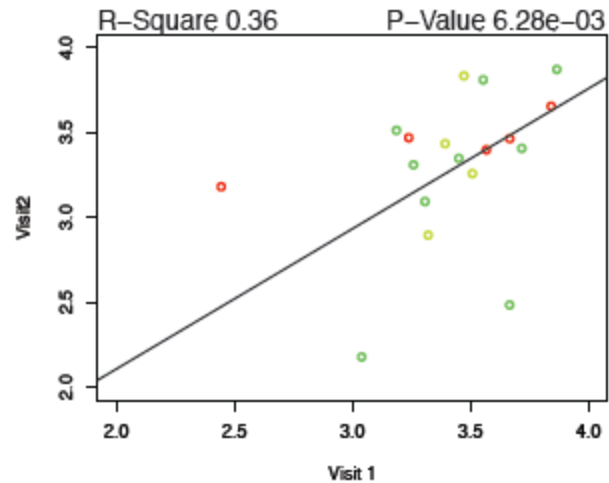
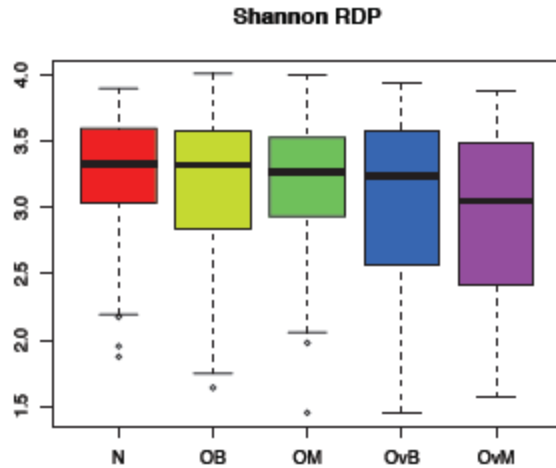


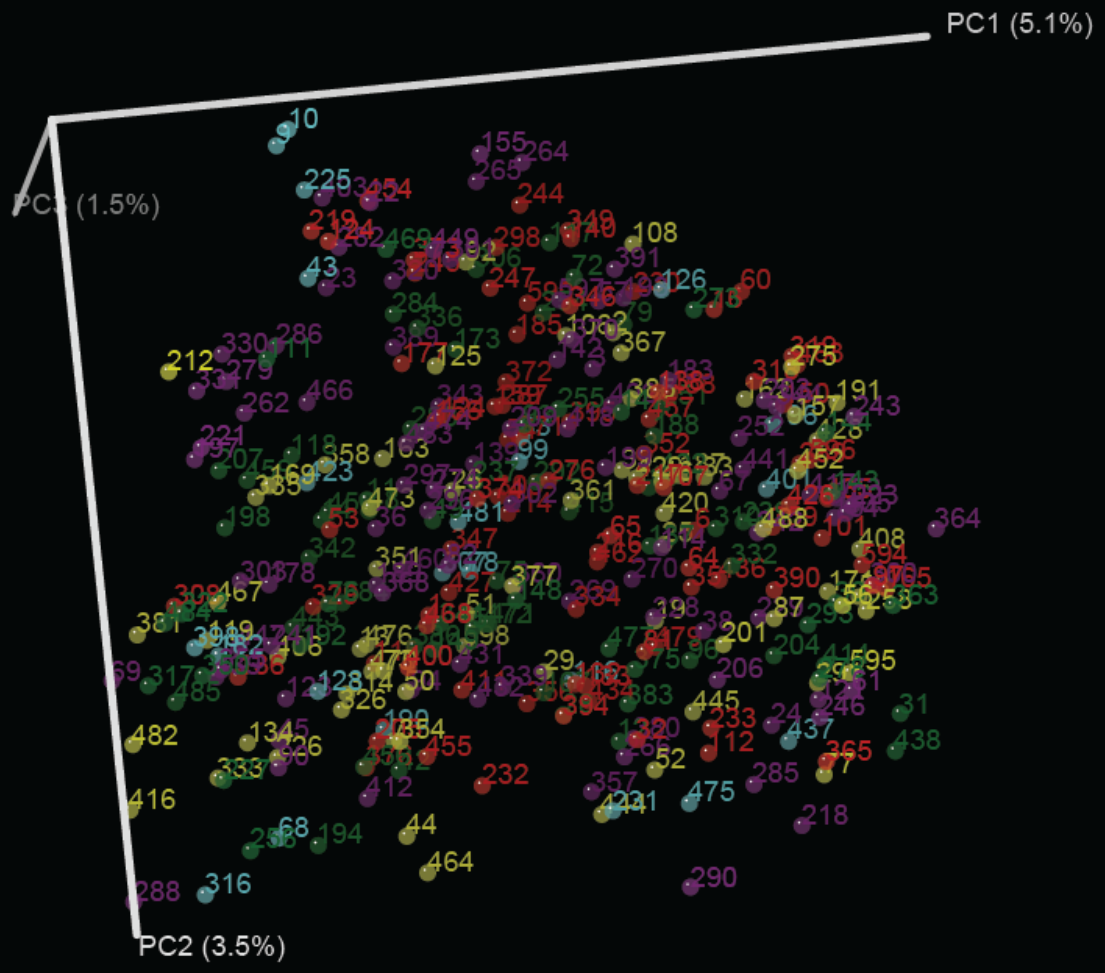
# Core Gut Microbiota

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- **Firmicutes (11)**
  - Clostridium
  - Eubacterium
  - Faecalibacterium
  - Ruminococcus
  - Oscillibacter
  - Lachnobacterium
  - Roseburia
  - Dorea
  - Coprococcus
  - Sporacetigenium
  - Blautia
- **Bacteroidetes (2)**
  - Bacteroides
  - Prevotella

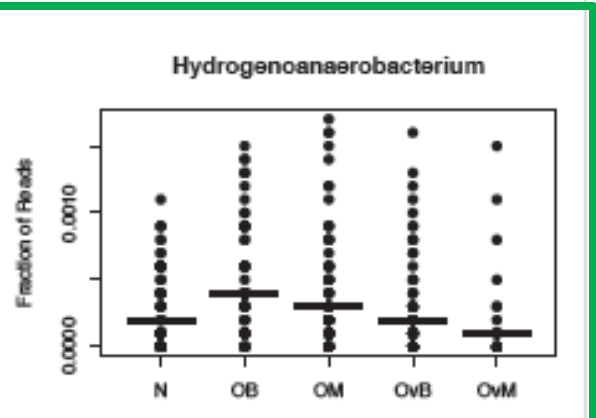
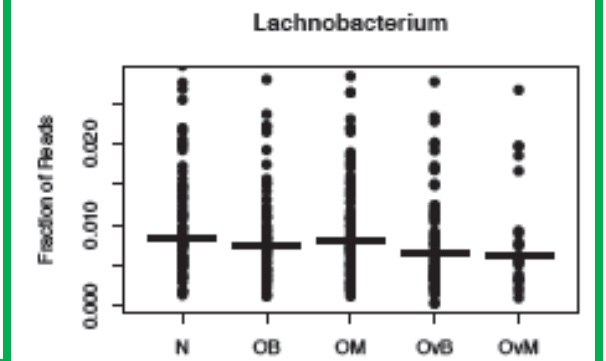
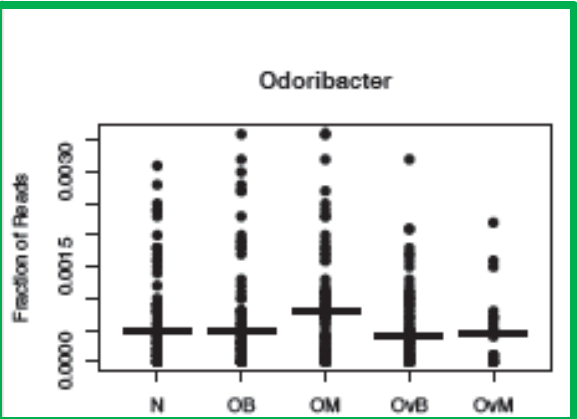
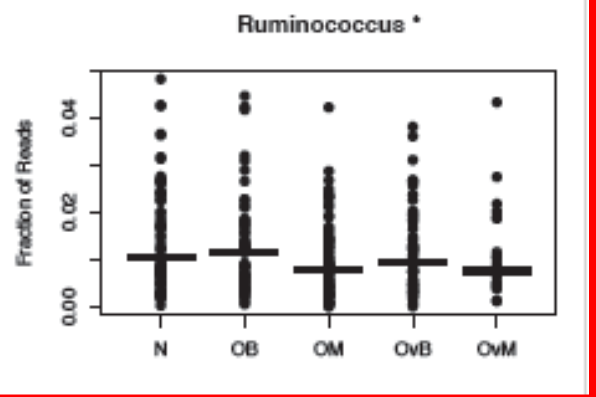
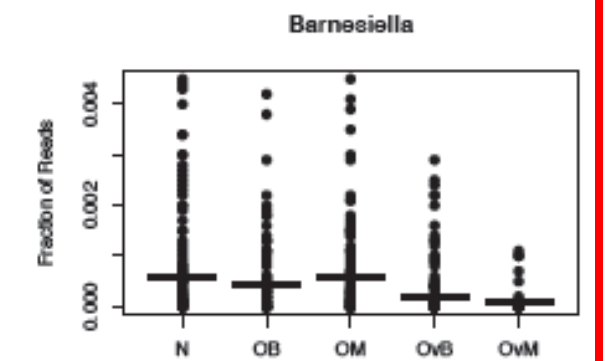
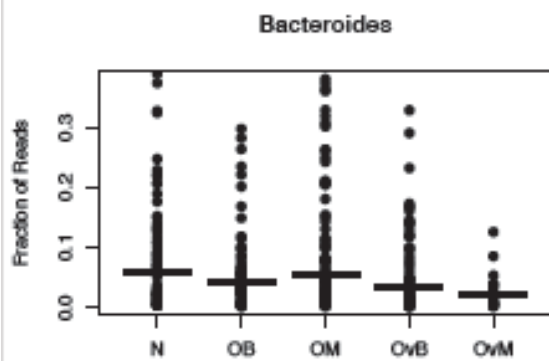
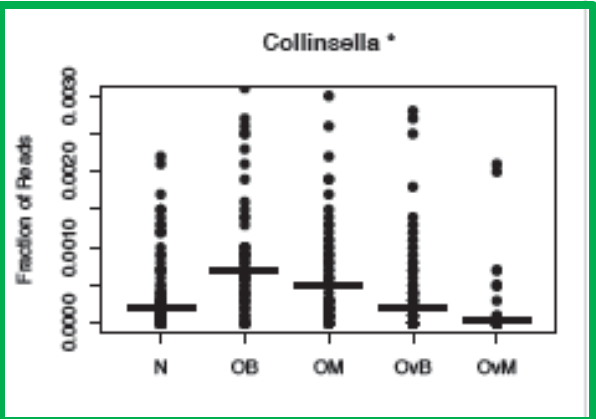
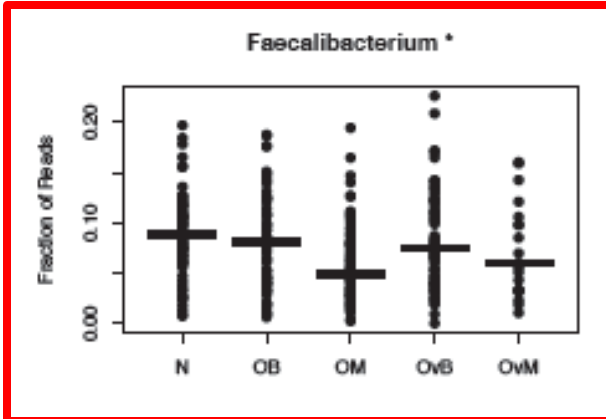
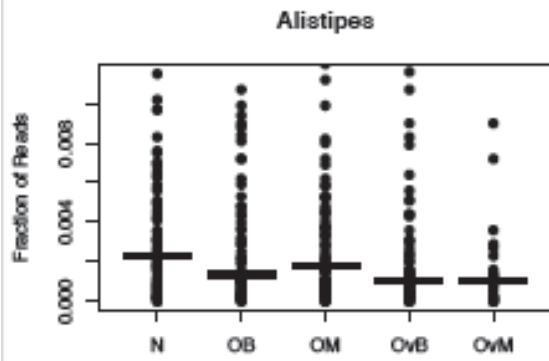
# Diversity of the Gut Microbiota





## Table 2. Household and Heritability Effect

	Heritability		Household Effect	
	$\beta \pm se$	p-value	$\beta \pm se$	p-value
Bacteroides: Firmicutes	$0.220 \pm 0.203$	0.24	$5.7 \times 10^{-4} \pm 1.2 \times 10^{-3}$	0.62
Shannon Index OTU	$0.263 \pm 0.206$	0.18	$-8.6 \times 10^{-4} \pm 2.5 \times 10^{-3}$	0.73
Shannon Index RDP	$0.237 \pm 0.207$	0.23	$-2.5 \times 10^{-4} \pm 3.4 \times 10^{-4}$	0.45

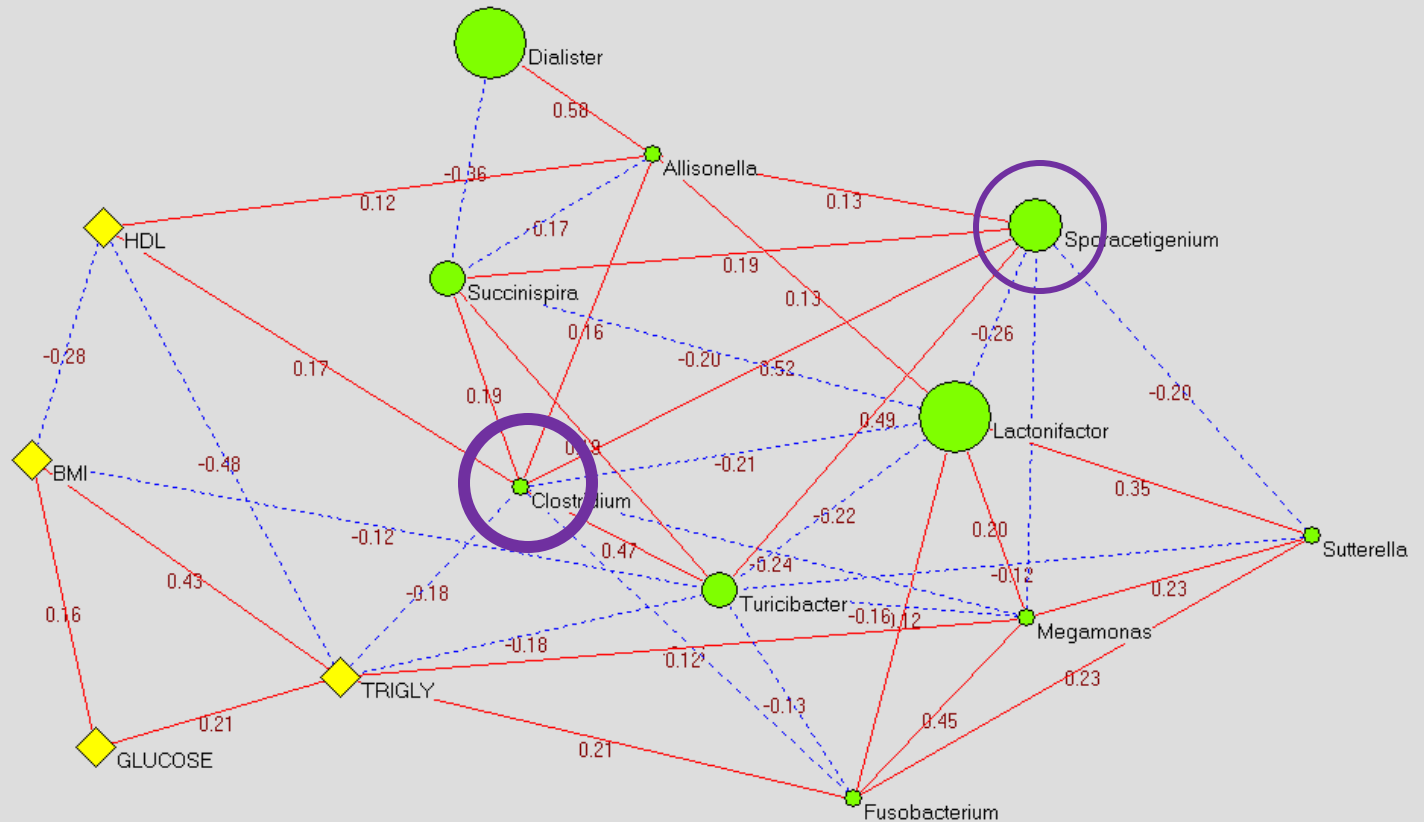




# Bacterial genera that are differentially abundant in lean vs. obese OOA

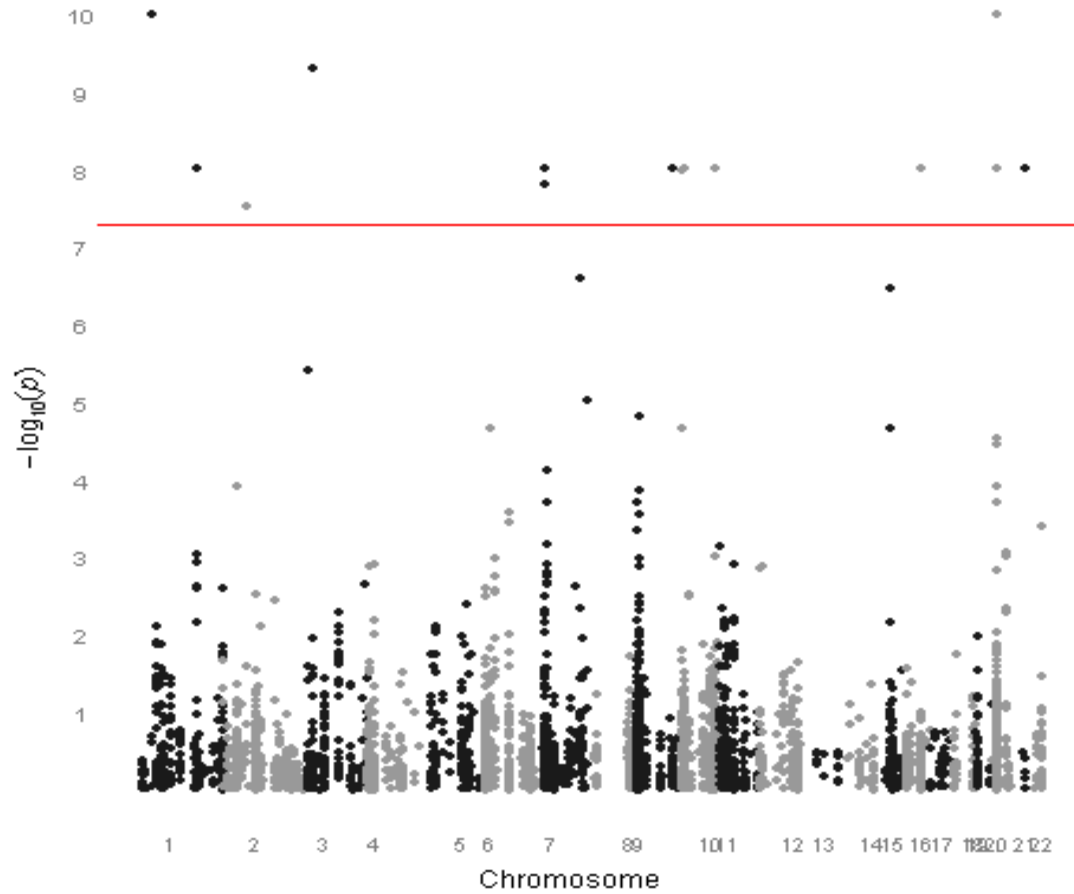
- *Ruminococcus*: Diet-responsive group of amylolytic bacteria that are increased in response to diets high in resistant starch [Walker et al. (2010) ISME J.]
- *Faecalibacterium*: An **anti-inflammatory** gut bacteria that is found in lower levels in patients with CD [Sokol et al. (2008) PNAS]

# Network analysis reveals eleven genera that are linked to serum triglyceride levels, cholesterol, and BMI ( $p < 0.05$ )

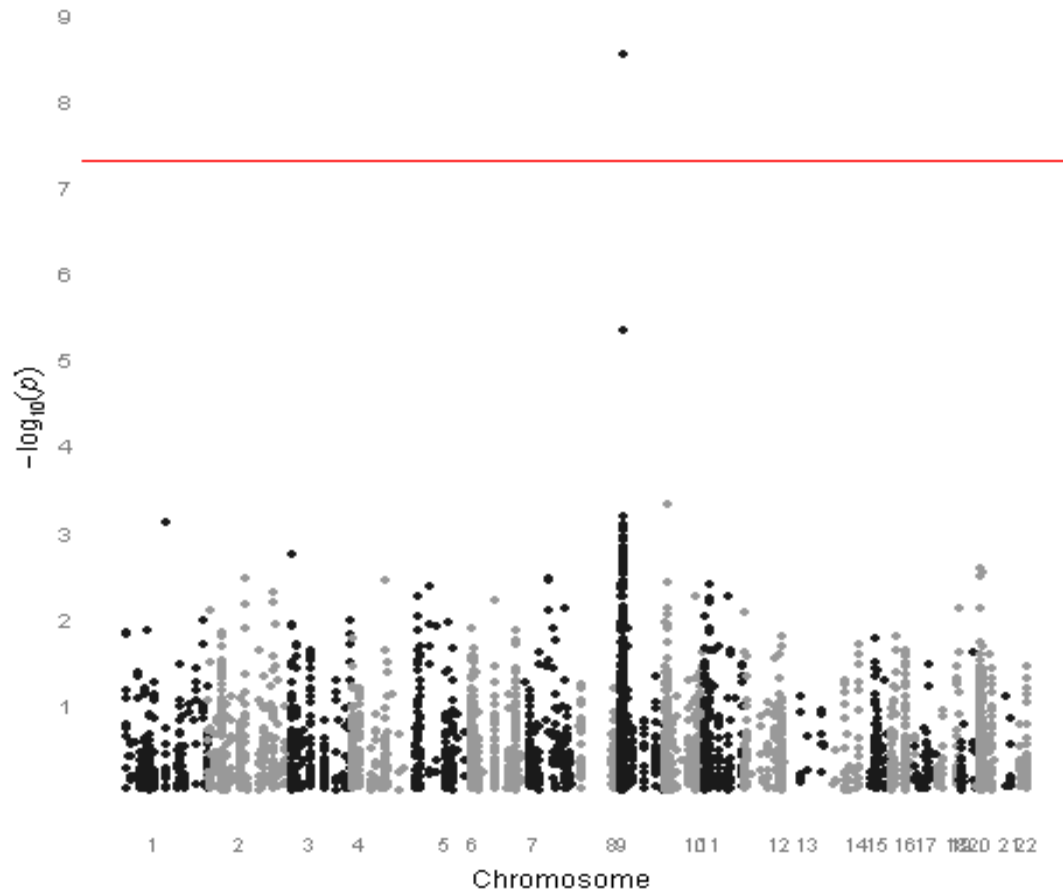




# FUSOBACTERIA



# FIRMICUTES



**16S rRNA analysis has  
revealed who's there**

**What are they doing?**

# Amish Thrifty Microbiome Project

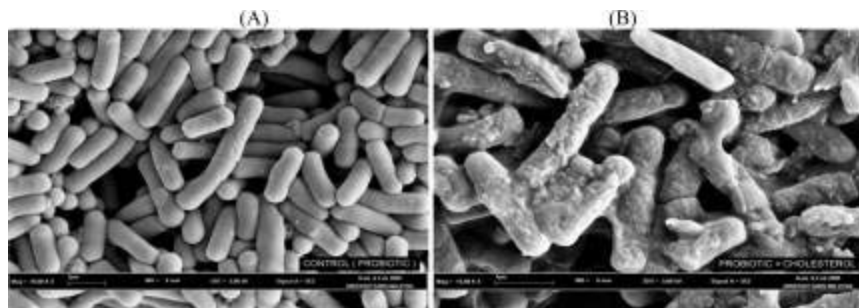
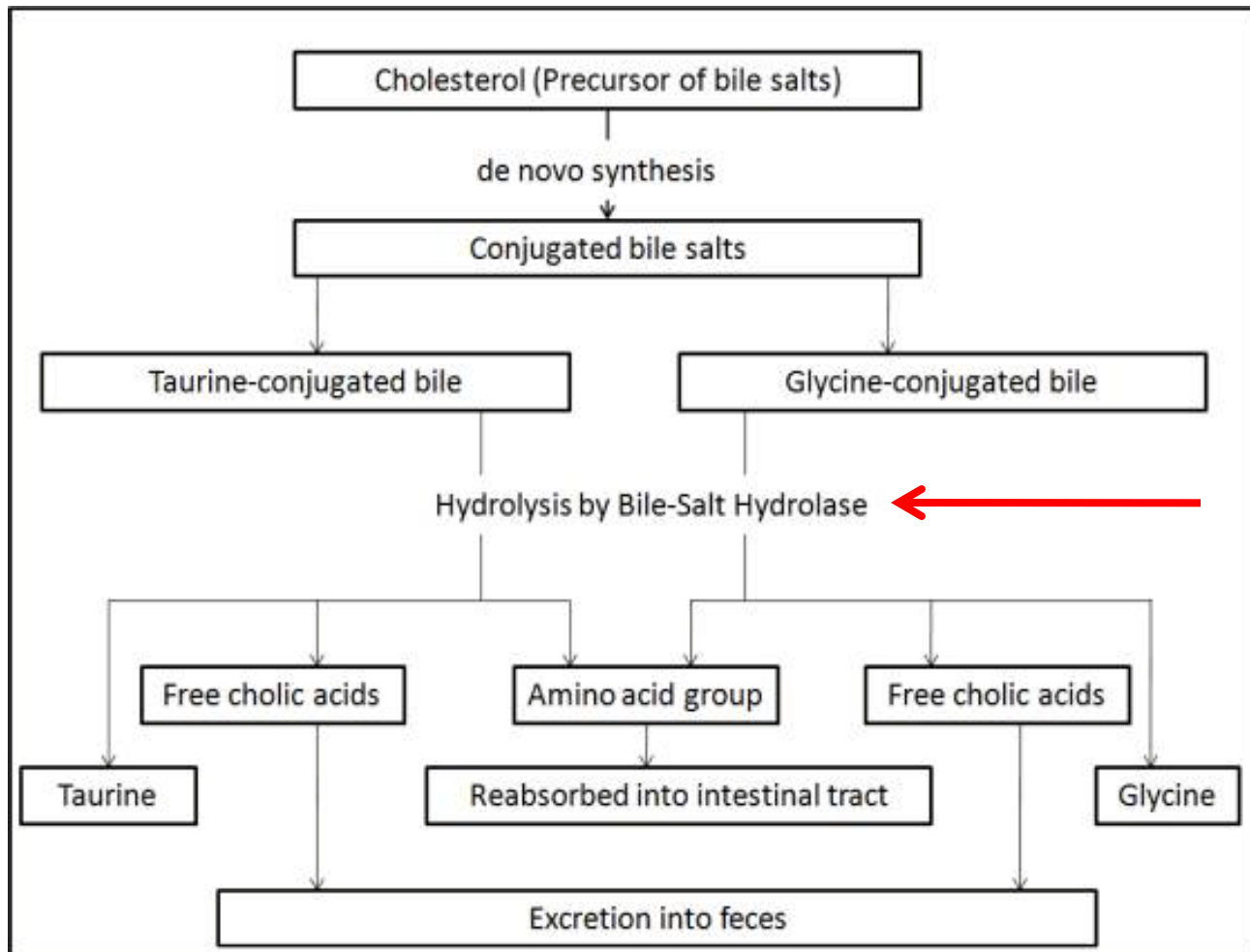
- Phase II – Metatranscriptomics
- Phase III – Intervention
  - Detailed metabolic characterization of subjects with metabolic syndrome
    - before and after treatment with neomycin
  - Host genome x gut microbiome interactions through GWAS



**Expected result:**

**Obese subjects with metabolic syndrome will exhibit a thrifty metabolism phenotype that will be reversed after treatment with antibiotics.**

**GWAS will identify novel genes and pathways that influence the gut microbiome and metabolic syndrome traits.**

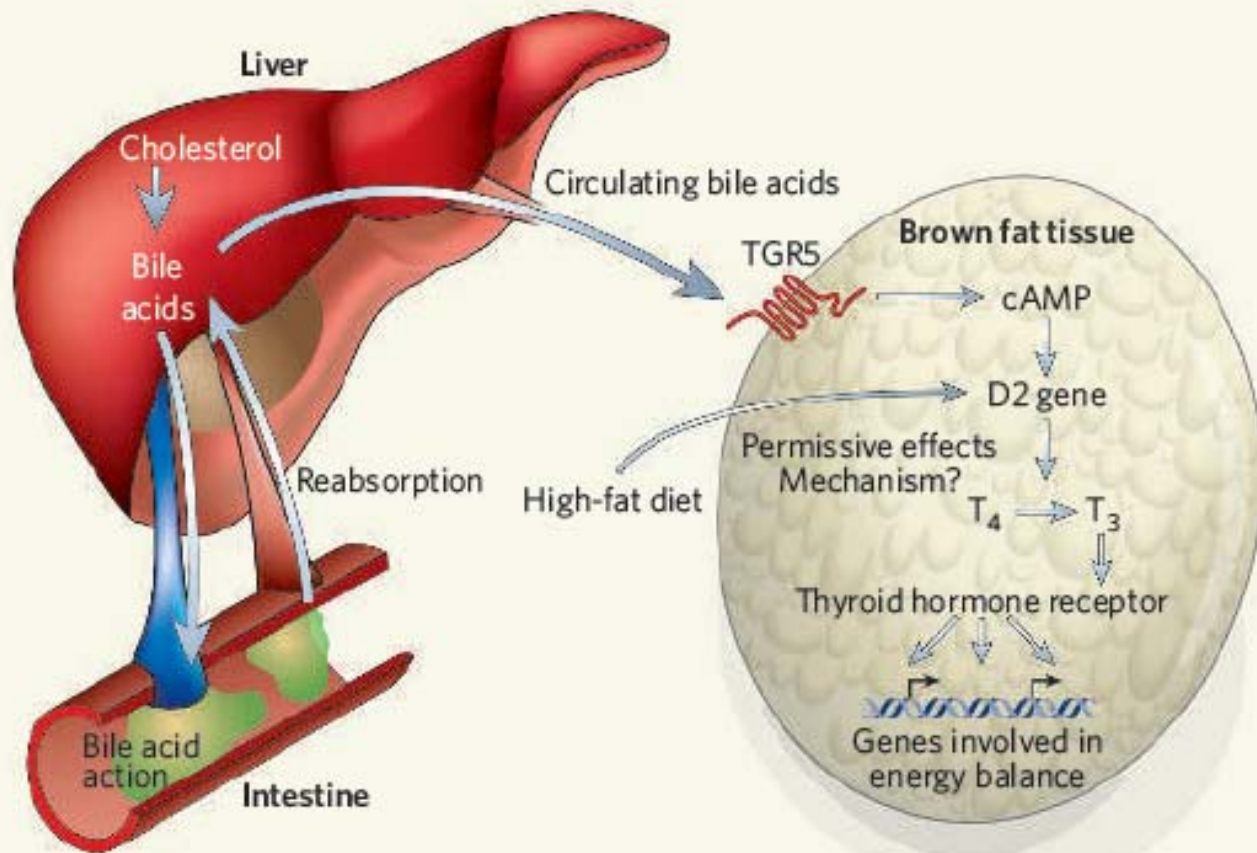


Cholesterol-Lowering Effects of Probiotics and Prebiotics: A Review of *in Vivo* and *in Vitro* Findings (2010)  
Lay-Gaik Ooi and Min-Tze Liong

# Bile salt transformation by anaerobic bacteria

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- *Eggerthella lenta*
- *Clostridium perfringens*
- *Peptostreptococcus spp.*
- *Bacteroides fragilis*
- *Bacteroides thetaiotaomicron*
- *Escherichia coli*
- *Eubacterium aurofaciens*
- *Clostridium spp.*
- *Clostridium absonum*
- *Clostridium sordelii*
- *Clostridium innocuum*
- *Clostridium scindens*
- *Clostridium bifermentans*
- *Clostridium leptum*



## METABOLISM

# Bile acids heat things up

John D. Baxter and Paul Webb

Thyroid hormone causes fat loss, but harnessing this action to treat obesity is difficult because it is associated with harmful side effects. However, bile acids generate active thyroid hormone just where it is needed.

# Metagenomic studies of the human gut in the Old Order Amish

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