

Cross-Study Evaluation of the Relationship between the Human Gut Microbiome and Heart Disease

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Introduction

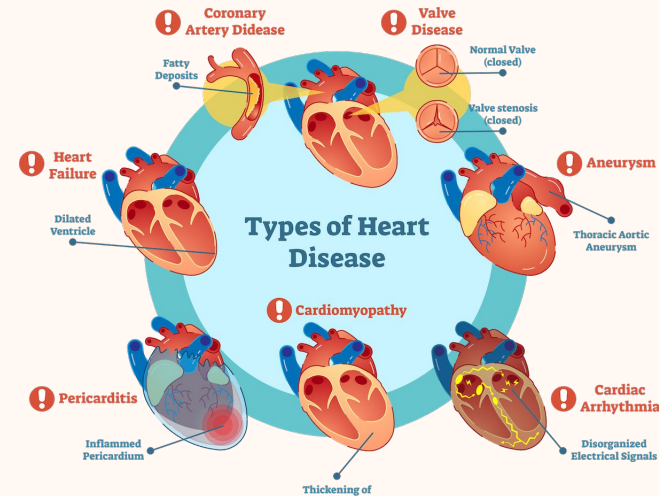
What is heart disease?



Introduction (cont.)

Heart disease refers to several types of heart conditions:

- “any disease that affects the heart or blood vessels”
- including but not limited to:
 - coronary artery disease (CAD)
 - cardiovascular disease
 - atherosclerosis



What is Atherosclerosis?

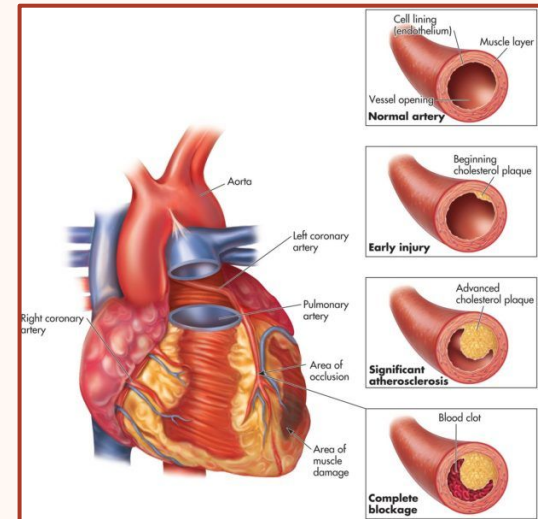
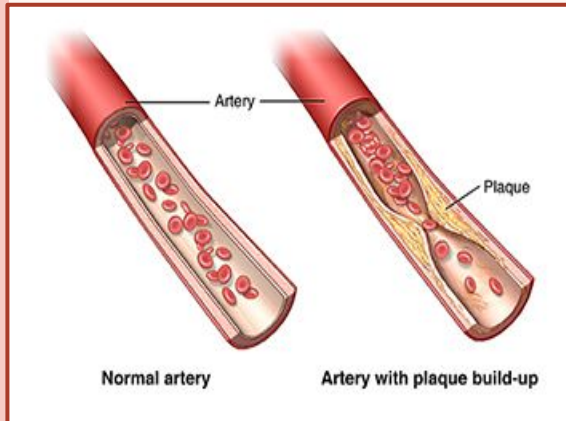
general term for the buildup of fat, cholesterol, etc (plaque) in the arteries



arteries narrow

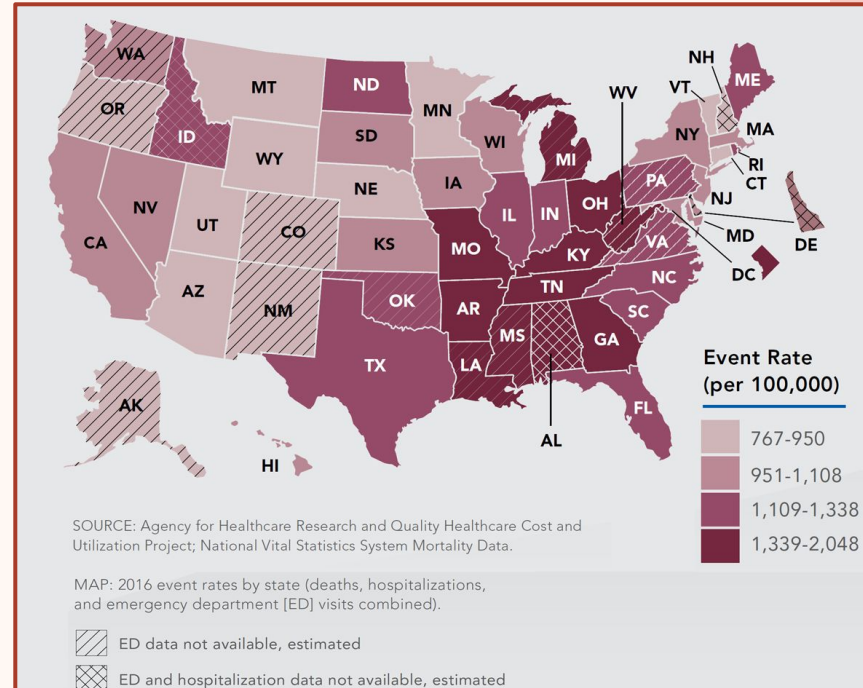


reduced blood flow



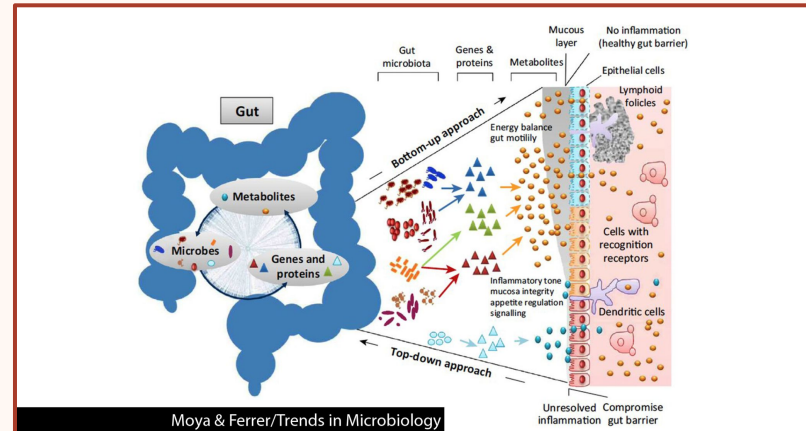
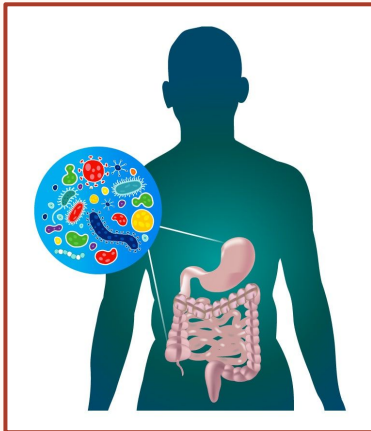
Why is it important?

- Cardiovascular disease is the number one cause of death worldwide
 - 17.9 million lives lost per year
- every 34 seconds, one person dies from heart disease in the USA
- 80% of premature heart disease and stroke cases are preventable



The Gut Microbiome

- Community of microorganisms that operate as a “hidden organ”
 - regulates healthy immune function
 - act as a bioreactor affecting several organ systems
 - metabolic engine
 - dynamic



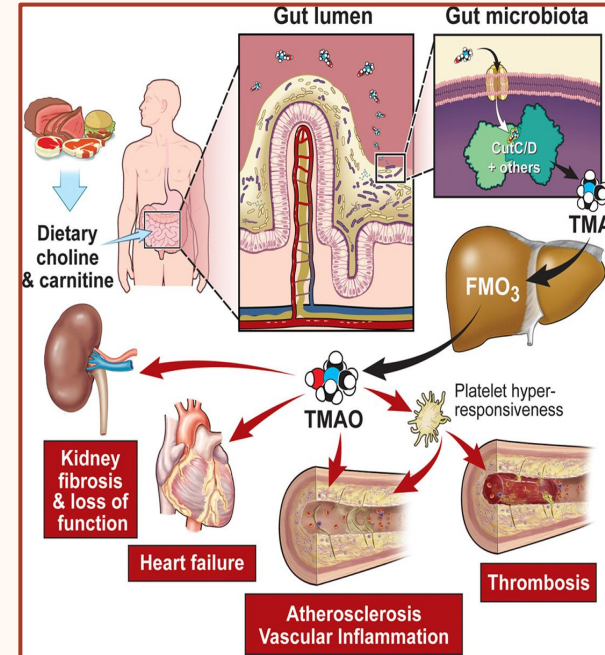
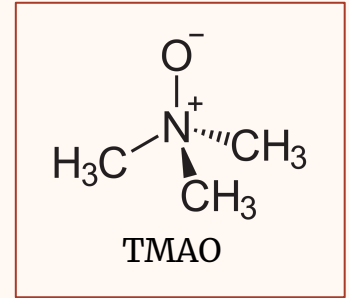
Background

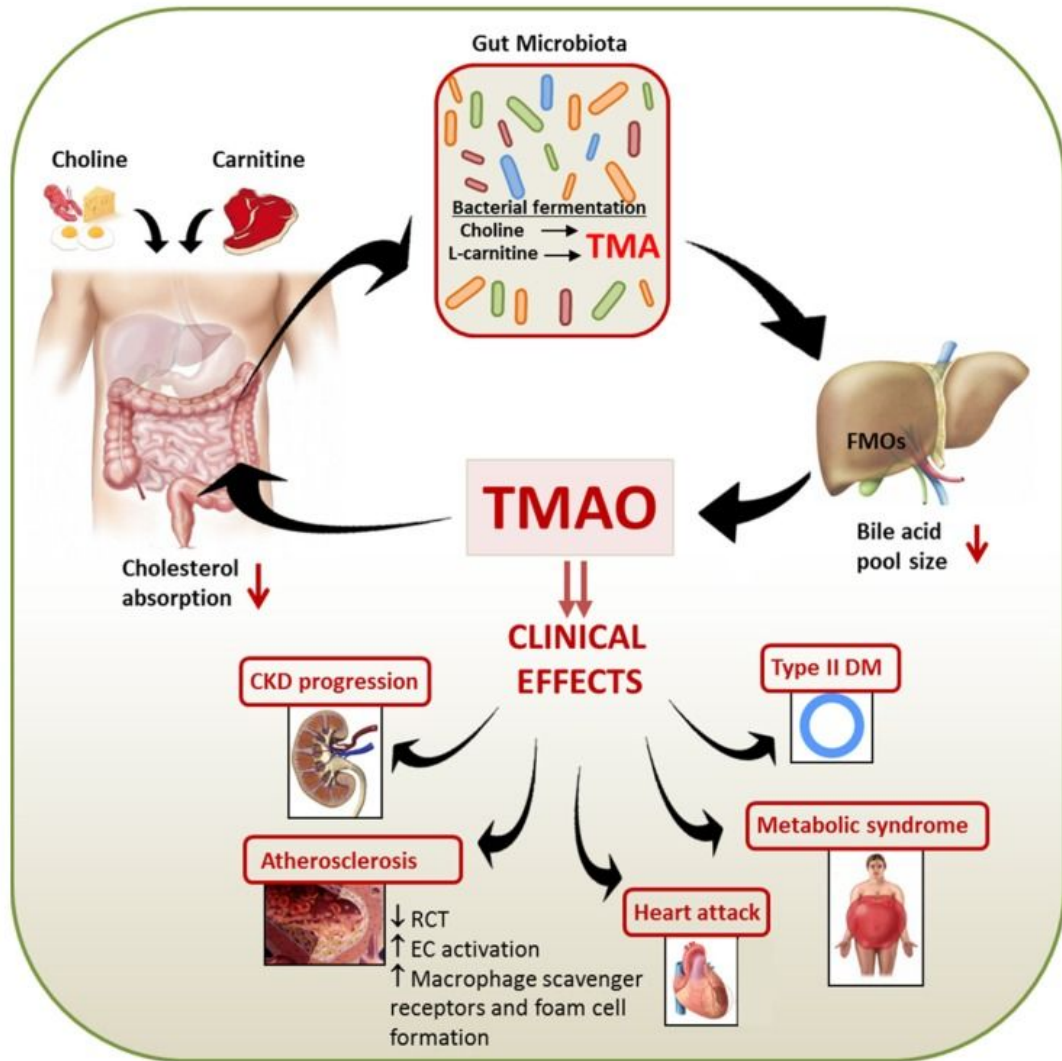
- Recent studies assessing relationship between atherosclerosis and the gut microbiome
- Working hypotheses about inflammation:
 - increased state of lipopolysaccharides (LPS)
 - trimethylamine N-oxide(TMAO)
 - short chain fatty acids
 - and bile acids

Background

TMA (trimethylamine) to TMAO (trimethylamine-N-oxide) pathway

- pathway is somewhat unclear but research shows:
 - dietary choline and carnitine → increased TMAO levels linked to heart failure and atherosclerosis via greater accumulation of bacterial products in the bloodstream
 - TMAO is a proinflammatory metabolite
 - associated with alterations in cholesterol and bile acid → inflammation → a leaky and unstable gut barrier*
 - Atherosclerotic plaques' bacterial DNA found in affected individuals' gut microbiome





Why Conduct a Meta-Analysis?


- Microbiome is rapidly evolving
- Currently, the field of microbiome research has many conflicting opinions
 - No consensus on best practices
- Heart Disease is a pivotal disease to understand and prevent
- Must give due credit to prior research and correct using modern day best practices



Aims

Aim 1 – What is the relationship between human gut microbiome and heart disease? For each individual study, are there new or updated biological findings that can be mined from the original when re-assessed using modern best-practices and databases?

Aim 2 (in progress) – What do the signals in the gut microbial communities of patients with heart disease tell us about potential interactions when evaluated across all existing manuscripts on the subject?



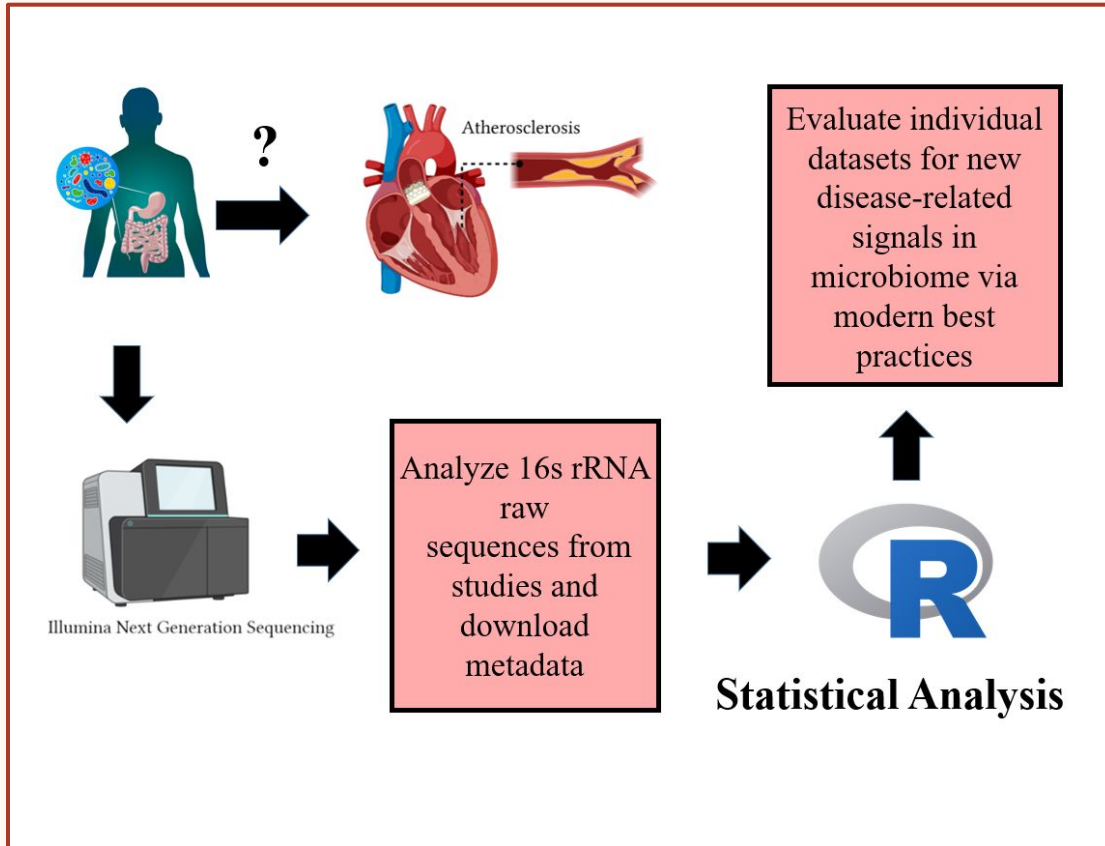
Hypothesis

We hypothesize that by applying cutting edge, modern bioinformatic approaches to microbiome analyses, we should be able to uncover new phenomena between heart disease and the microbiome, specifically regarding inflammation as a pivotal byproduct.

* This project will focus on assessing the role of microbial taxa, and thus by proxy, the microbial metabolites.

- biases
- newer databases

Materials and Methods



Materials and Methods

Dysbiosis of Gut Microbiota With Reduced Trimethylamine-N-Oxide Level in Patients With Large-Artery Atherosclerotic Stroke or Transient Ischemic Attack

[Jia Yin](#), MD, ¹ [Shuo-Xi Liao](#), MS, ¹ [Yan He](#), MD, ³ [Shan Wang](#), MS, ³ [Geng-Hong Xia](#), MS, ¹ [Fei-Tong Liu](#), MS, ³ [Jia-Jia Zhu](#), MD, ¹ [Chao You](#), MS, ¹ [Qiong Chen](#), MS, ¹ [Liang Zhou](#), MD, ¹ [Su-Yue Pan](#), MD, ¹ and [Hong-Wei Zhou](#), PhD^{2,3}

The gut microbiome modulates the protective association between a Mediterranean diet and cardiometabolic disease risk

[Dong D Wang](#) ^{1 2}, [Long H Nguyen](#) ^{3 4}, [Yanping Li](#) ², [Yan Yan](#) ⁵, [Wenjie Ma](#) ^{3 4}, [Ehud Rinott](#) ⁶, [Kerry L Ivey](#) ^{2 7 8}, [Iris Shai](#) ⁶, [Walter C Willett](#) ^{1 2 9}, [Frank B Hu](#) ^{1 2 9}, [Eric B Rimm](#) ^{1 2 9}, [Meir J Stampfer](#) ^{1 2 9}, [Andrew T Chan](#) ^{1 3 4}, [Curtis Huttenhower](#) ^{10 11}

Gut microbiota induces high platelet response in patients with ST segment elevation myocardial infarction after ticagrelor treatment

[Xi Zhang](#),^{#1,2,†} [Xiaolin Zhang](#),^{#1,†} [Fangnian Tong](#),¹ [Yi Cai](#),¹ [Yujie Zhang](#),¹ [Haixu Song](#),¹ [Xiaoxiang Tian](#),¹ [Chenghui Yan](#),¹ and [Yaling Han](#),^{1,2}

Arduino A Mangoni, Reviewing Editor and Matthias Barton, Senior Editor
Arduino A Mangoni, Flinders Medical Centre Australia ;

Gut Microbiome Associates With Lifetime Cardiovascular Disease Risk Profile Among Bogalusa Heart Study Participants

[Tanika N Kelly](#) ¹, [Lydia A Bazzano](#) ², [Nadim J Ajami](#) ², [Hua He](#) ², [Jinying Zhao](#) ², [Joseph F Petrosino](#) ², [Adolfo Correa](#) ², [Jiang He](#) ²

Symptomatic atherosclerosis is associated with an altered gut metagenome

[Fredrik H Karlsson](#) ¹, [Frida Fåk](#), [Intawat Nookaew](#), [Valentina Tremaroli](#), [Björn Fagerberg](#), [Dina Petranovic](#), [Fredrik Bäckhed](#), [Jens Nielsen](#)

The gut microbiome in atherosclerotic cardiovascular disease

[ZhuYe Jie](#) ^{1 2 3}, [Huihua Xia](#) ^{1 2}, [Shi-Long Zhong](#) ^{4 5}, [Qiang Feng](#) ^{1 2 6 7 8}, [Shenghui Li](#) ¹, [Suisha Liang](#) ^{1 2}, [Huanzi Zhong](#) ^{1 2 3 7}, [Zhipeng Liu](#) ^{1 9}, [Yuan Gao](#) ^{1 2}, [Hui Zhao](#) ¹, [Dongya Zhang](#) ¹, [Zheng Su](#) ¹, [Zhiwei Fang](#) ¹, [Zhou Lan](#) ¹, [Junhua Li](#) ^{1 2 3 10}, [Liang Xiao](#) ^{1 2 6}, [Jun Li](#) ¹, [Ruijun Li](#) ¹¹, [Xiaoping Li](#) ^{1 2}, [Fei Li](#) ^{1 2 9}, [Huahui Ren](#) ¹, [Yan Huang](#) ¹, [Yangqing Peng](#) ^{1 12}, [Guanglei Li](#) ¹, [Bo Wen](#) ^{1 2}, [Bo Dong](#) ¹, [Ji-Yan Chen](#) ⁴, [Qing-Shan Geng](#) ⁴, [Zhi-Wei Zhang](#) ⁴, [Huanming Yang](#) ^{1 2 13}, [Jian Wang](#) ^{1 2 13}, [Jun Wang](#) ^{1 14 15}, [Xuan Zhang](#) ¹⁶, [Lise Madsen](#) ^{1 2 7 17}, [Susanne Brix](#) ¹⁸, [Guang Ning](#) ¹⁹, [Xun Xu](#) ^{1 2}, [Xin Liu](#) ^{1 2}, [Yong Hou](#) ^{1 2}, [Huijue Jia](#) ^{20 21 22 23}, [Kunlun He](#) ²⁴, [Karsten Kristiansen](#) ^{25 26 27}

Alterations of Gut Microbiome in Tibetan Patients With Coronary Heart Disease

 [Fengyun Liu](#)^{1,2†},  [Chao Fan](#)^{3,4†},  [Liangzhi Zhang](#)^{3†},  [Yuan Li](#)^{1,2},  [Haiwen Hou](#)^{1,2},  [Yan Ma](#)^{1,2},  [Jinhua Fan](#)^{1,2},  [Yueqin Tan](#)^{1,2},  [Tianyi Wu](#)^{1,2†},  [Shangang Jia](#)^{5†} and  [Yanming Zhang](#)^{3†}

Elevated Levels of Circulating DNA in Cardiovascular Disease Patients: Metagenomic Profiling of Microbiome in the Circulation

[Vasudevan Dinakaran](#), ¹ [Andiappan Rathinavel](#), ² [Muthuirulan Pushpanathan](#), ¹ [Ramamoorthy Sivakumar](#), ¹ [Paramasamy Gunasekaran](#), ¹ and [Jeyaparakash Rajendhran](#) ^{1, *}

Statistical Analysis

1. Collect all available primary studies on the gut microbiome and heart disease

- Sources: PubMed, NCBI, etc
- Make sure the raw sequencing data is available as 16s rRNA, along with metadata

2. Download each individual dataset and its metadata

- Use best modern day approaches to download data (fastq files) via the Linux terminal

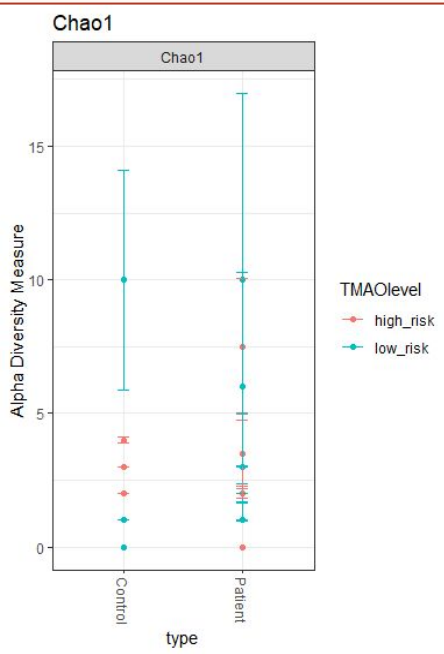
3. * Evaluate each individual dataset for new disease-related signals in the microbiome via modern best practices

- Intra Study analysis
 - R studio
 - Dada2
 - CutAdapt
 - ASV table
 - Taxonomy table
 - etc.

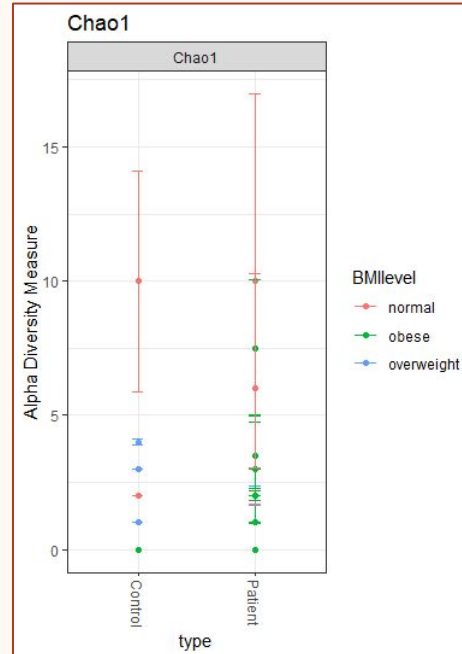
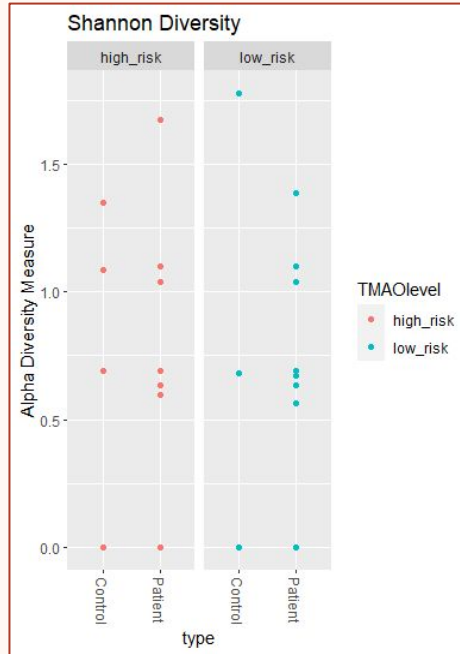
4. Compare datasets and make conclusions

- Merging functions in phyloseq
- Create 1 unified dataset

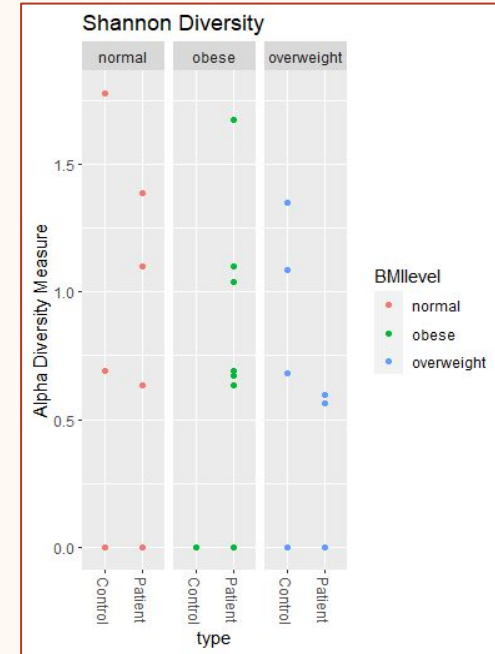
Results in Progress



assessing TMAO Level with patient type



assessing BMI Level with patient type



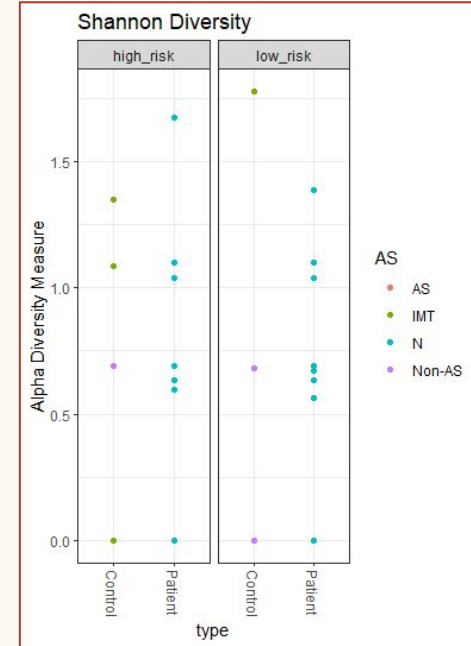
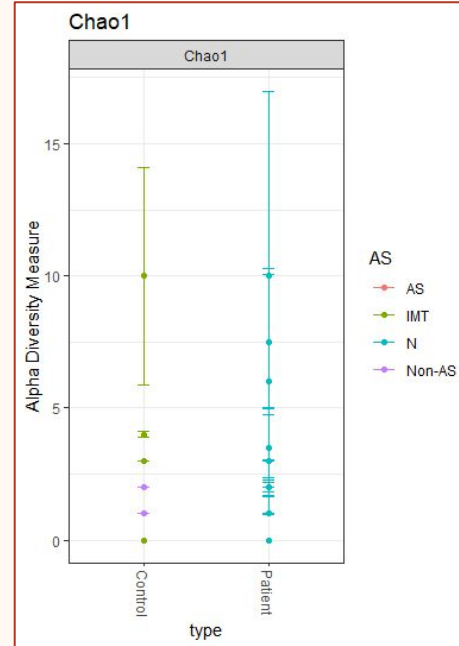
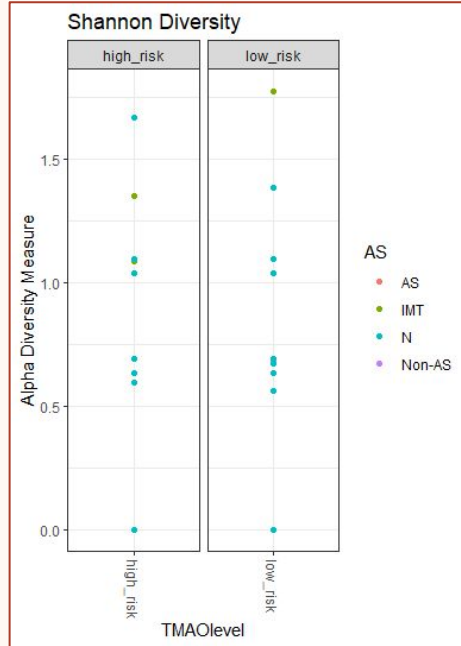
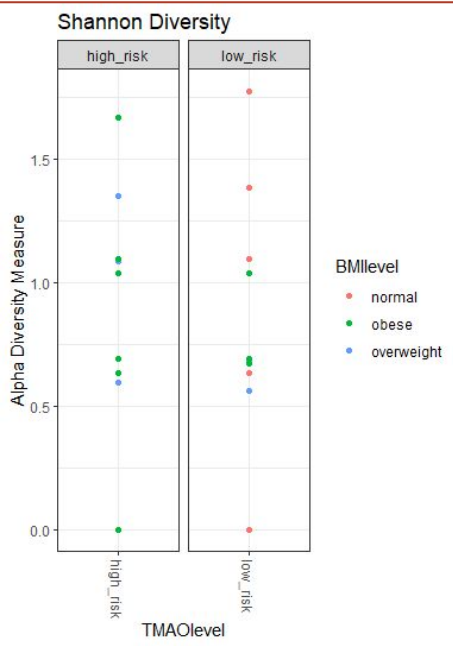
α Diversity Plots

Chao: species richness

Observed Species: number of unique OTU/distinct taxa

Shannon: measures species richness and diversity

Results in Progress

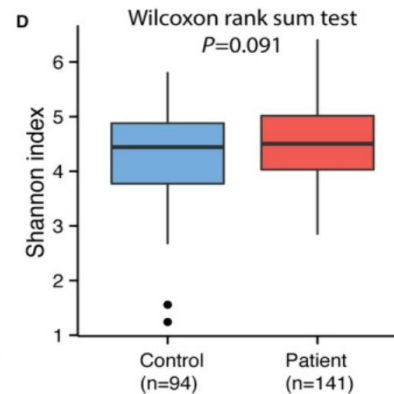
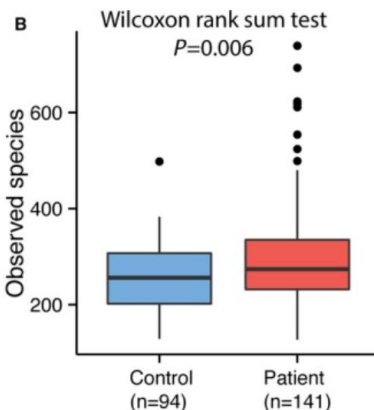
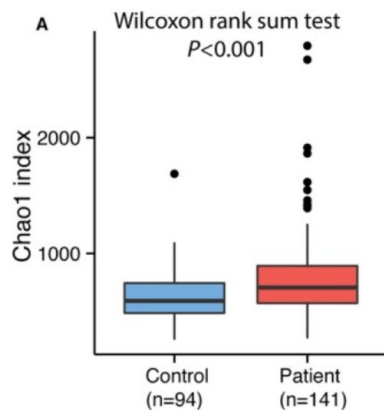


α Diversity Plots

Results In Progress

Alpha diversity plots
from Yin et. al
2015

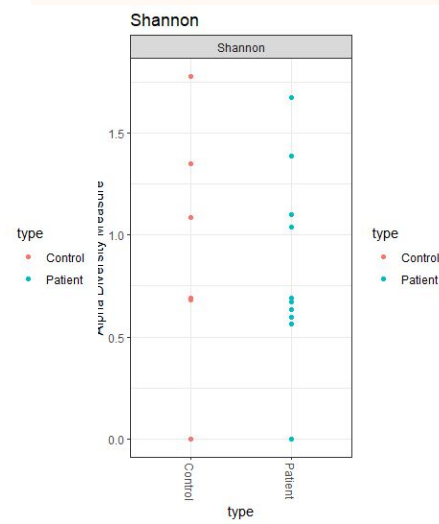
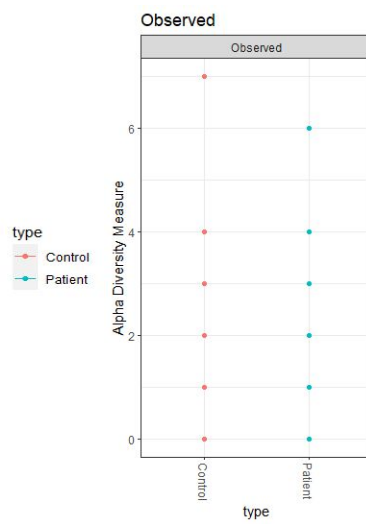
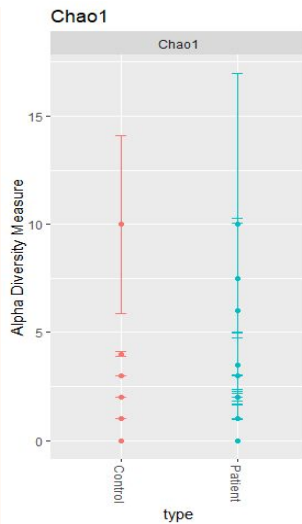
QIIME pipeline



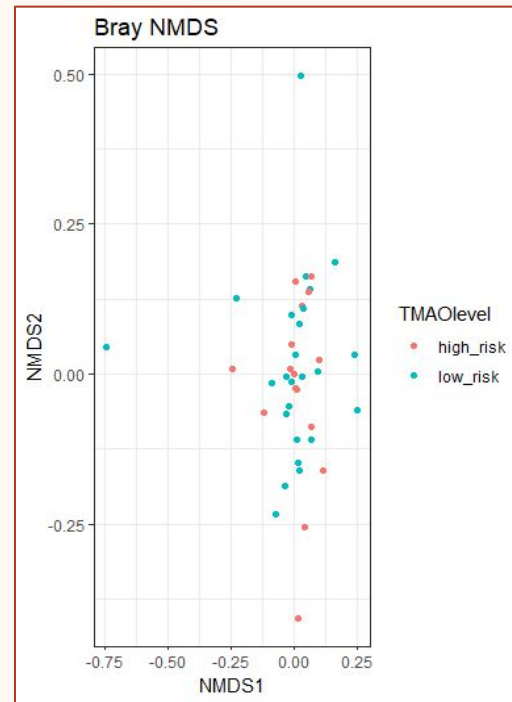
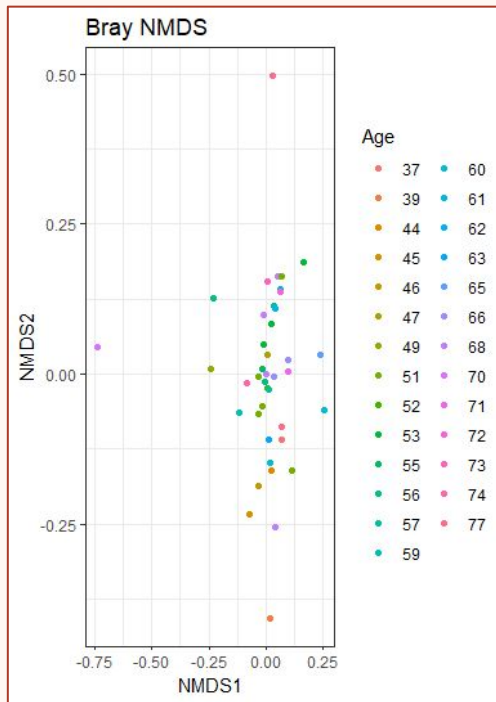
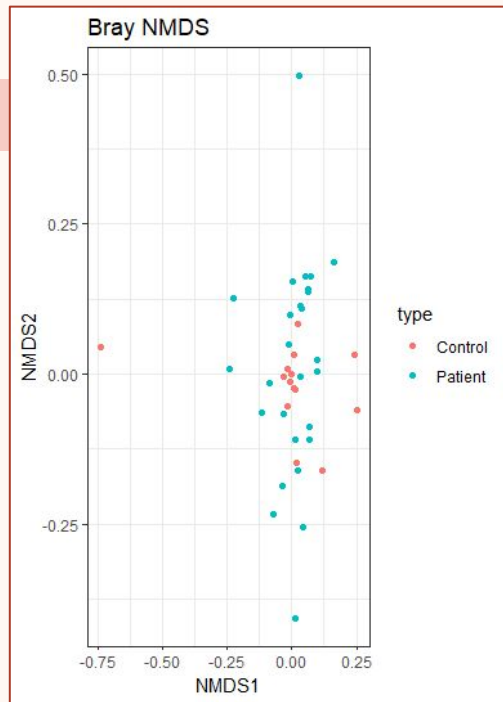
Chao: species richness
Observed Species: number of unique OTU/distinct taxa
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Alpha diversity plots
generated in 2022-23

dada2 pipeline

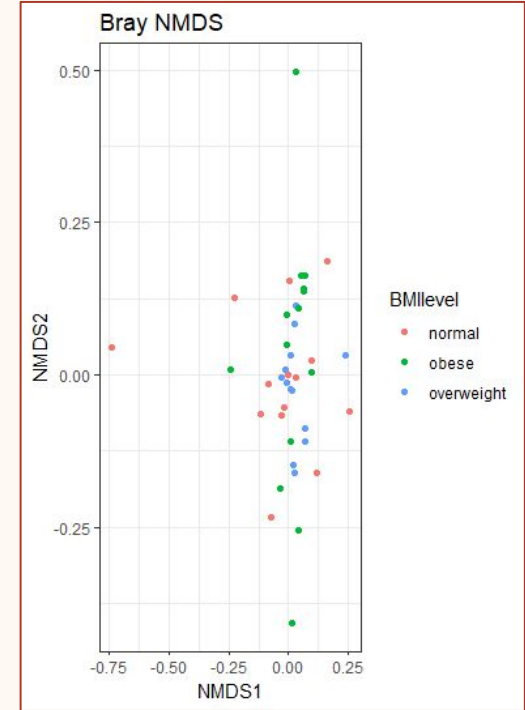
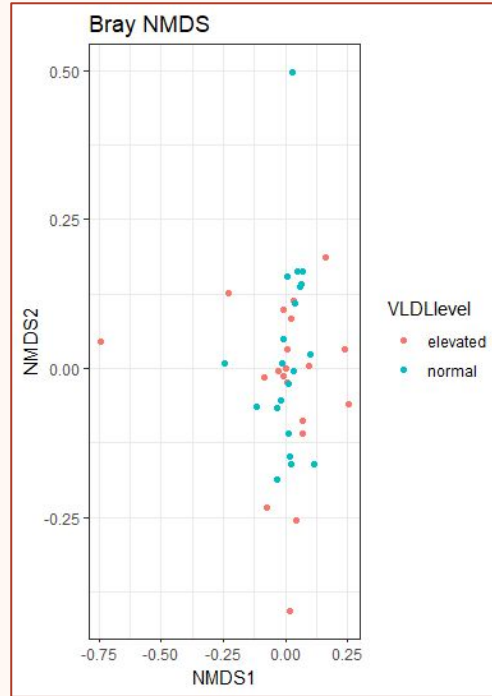
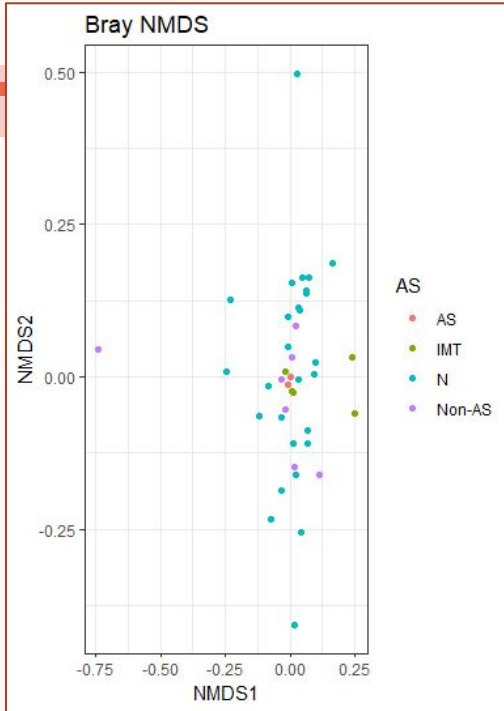


Results in Progress



β Diversity Plots

Results in Progress



β Diversity Plots

Discussion in Progress

For this study:

- Data is uninterpretable
 - Not replicable
 - Unclear
 - No raw data provided
- 2 main issues
 - True raw sequencing was not provided
 - Data that has been re-assessed is not truly valid
- there are some convoluted data to be further examined
 - cited 453 times

Next Steps

- request a grant to re-do study
 - however, unlikely to be funded due to misunderstandings
 - difficult to dislodge
- Continue statistical analysis for other studies listed
- Compile a larger set of data and complete a cross-study analysis to identify microbial taxa involved in heart disease

Acknowledgements

Thank you to Dr. Michael B. Burns and Loyola University Chicago for aiding and funding this project.

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