# The relationship between alcohol consumption and gut microbiome diversity in individuals with AUD and controls

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## Background

- Alcohol use disorder (AUD) is associated with alterations in the composition and function of the gut microbiome
- It is unknown whether alcohol-associated gut dysbiosis is more influenced by:
  - recent alcohol consumption or chronic alcohol use
  - the quantity of alcohol consumed or the presence of an AUD

**Aims:** (1) Explore the relationship between gut microbial dysbiosis and AUD

(2) Investigate the relationship between gut dysbiosis and alcohol consumption

| | AUD | Control |
|---|---|---|
| Age | 45.48 | 50.42 |
| % Women | 39.13 | 15.79 |
| BMI | 29.30 | 27.13 |
| AUDIT | 16.80 | 3.11 |
| Total Drinks per 30 days | 205.78 | 13.78 |
| Heavy Drinking Days per 30 days | 15.65 | 0.63 |
| Days since last drink | 2.23 | 8.38 |
| Heavy Drinking Days = ≥5/≥4 drinks per day for men/women | | |

## Methods

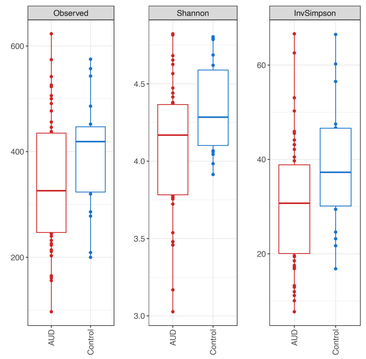
- Participants were recruited from the general population and divided into two groups:
  - **AUD** (n = 46): Actively drinking, 25-68 years old with DSM-5 AUD
  - **Controls** (n = 19): No DSM-5 disorders
- Stool samples were collected at home, returned to the laboratory, and stored in -80C freezers until sequencing
- 16s rRNA gene sequencing was performed using the Illumina NextSeq 550 platform
- Alpha and beta diversity determined using the phyloseq package in R
  - ***Alpha diversity***: Richness (number of taxa present) and evenness (how taxa are distributed) within a sample was determined by the number of observed species, the Shannon index, and the inverse Simpson index
  - ***Beta diversity:*** Differences in overall taxonomic composition across different samples was determined using Jensen-Shannon divergence
- Taxa differences were assessed using the MaAslin2 package
- ***30-day Timeline Follow Back*** - measured alcohol consumption, including days
- ***The Alcohol Use Disorder Identification Test (AUDIT)*** – 10-item screening tool to identify problematic alcohol use, accounting for alcohol consumption and alcohol-related problems
- All analyses controlled for sex and BMI

## Results

### Diversity

**Figure 1: AUD vs. Controls**

1A. Alpha Diversity




1B. Beta Diversity

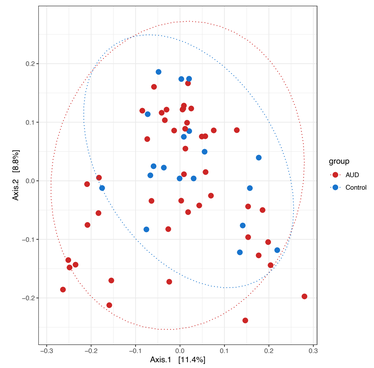
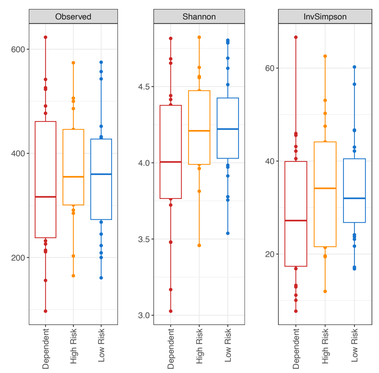
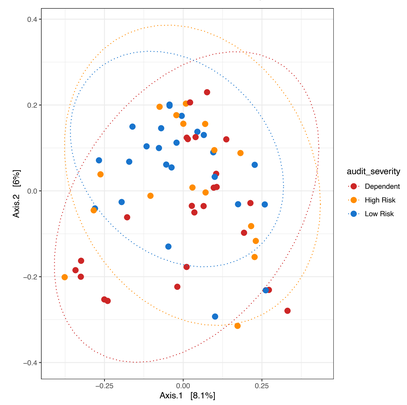



**Figure 2: AUDIT Scores**

2A. Alpha Diversity




2B. Beta Diversity

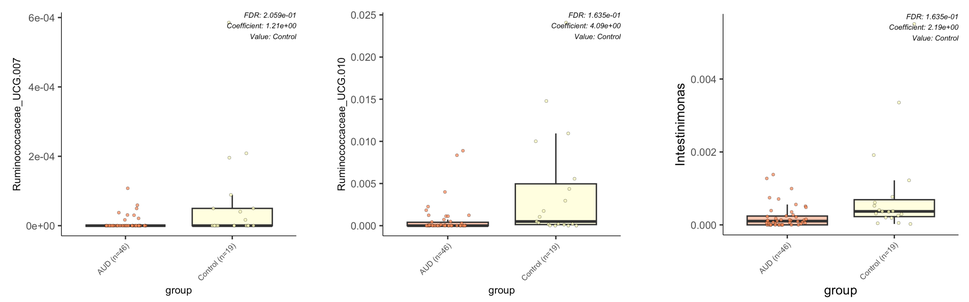



- **AUD diagnosis vs. controls**
  - AUD participants had significantly higher alpha diversity as measured by the Shannon (β=0.29, t=2.66, p<0.01) and inverse Simpson (β=8.03, t=2.14, p=0.037) indexes.
  - Beta diversity as measured by the Jensen-Shannon divergence was significantly different between groups ($R^2$=0.02, p=0.047)
- **AUDIT severity**
  - Individuals with greater AUDIT severity vs low risk drinkers had a trend toward reduced alpha diversity on the Shannon index (β=0.20, t=1.74, p=0.08) but not other indices.
  - Beta diversity did not differ between AUDIT severity groups ($R^2$=0.03,p=0.22)
- **Recent Alcohol Consumption**
  - Although alpha diversity was not associated with the 30-day total drinks (Observed [p=0.53], Shannon [p=0.74], InvSimpson [p=0.77]), total drinks explained a significant portion of variance in taxonomic composition ($R^2$=0.03, p=0.014).
  - Among individuals with AUD, but not controls, recent drinking was associated with greater alpha (Shannon [β=-0.11, t=-2.74, p=0.011]; InvSimpson [β=-2.91, t=-2.66, p=0.013]) but not beta diversity ($R^2$=0.05, F=p=0.09).

### Taxa differences between groups

**Figure 3: Differentially Abundant Taxa Between Groups**

3A. Genus Level




3B. Family Level

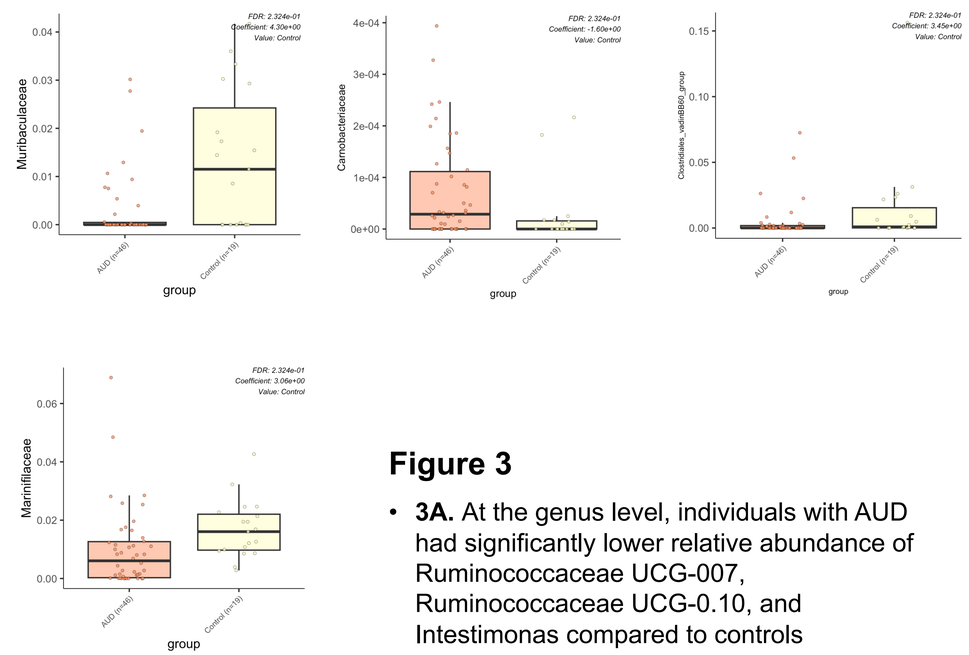



**Figure 3**

- **3A.** At the genus level, individuals with AUD had significantly lower relative abundance of Ruminococcaceae UCG-007, Ruminococcaceae UCG-0.10, and Intestimonas compared to controls
- **3B.** At the family level, individuals with AUD had significantly lower relative abundance of Muribaculaceae, Clostridiales vadin BB60, and Marinifilaceae. Individuals with AUD had a higher relative abundance of Carnobacteriaceae compared to controls.

## Conclusions

❖ Individuals with AUD vs. Controls show reduced alpha diversity and altered microbial composition, but this difference was not driven by differences in AUD severity.

❖ Greater quantities of alcohol consumed over 30 days was associated with differences in the composition of the gut microbiome but was not associated with alpha diversity

❖ In individuals with AUD only, recent drinking was associated with greater alpha diversity but not beta diversity.

❖ AUD was associated with reduced abundance in taxa associated with SCFA production (Ruminococcaceae UCG-007, Ruminococcaceae UCG-0.10), butyrate production (Intestimonas), the breakdown of complex carbohydrates (Muribaculaceae), gut health (Clostridiales vadin BB60), and liver fibrosis (Marinifilaceae).

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