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Any alcohol use in NAFLD patients is associated with significant changes to the intestinal virome

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Abstract

The prevalence of alcohol use disorder (AUD) and metabolic dysfunction-associated fatty liver disease (MAFLD) are increasing worldwide, leading to the increasing likelihood of both etiologies contributing to a patient's liver disease. However, the effects of modest alcohol use in non-alcoholic fatty liver disease (NAFLD) are controversial and more studies are needed. We compared the intestinal viromes of patients with AUD and NAFLD and evaluated the effect of alcohol consumption on the intestinal viromes of NAFLD patients by extracting virus-like particles and performing metagenomic sequencing. We demonstrate significant differences in the intestinal viromes of NAFLD and AUD patients, and that alcohol use in NAFLD patients reclassified to MAFLD accounted for significant differences in the intestinal viromes. The relative abundance of several *Lactococcus* phages was more similar between AUD patients and alcohol-consuming MAFLD patients than non-alcohol-consuming MAFLD patients and control subjects, and multivariate modeling using the most discriminating *Lactococcus* phages could better predict alcohol use in the MAFLD population than the ALD/NAFLD Index. Significant differences in the viral composition and diversity were also seen between MAFLD patients with low and moderate

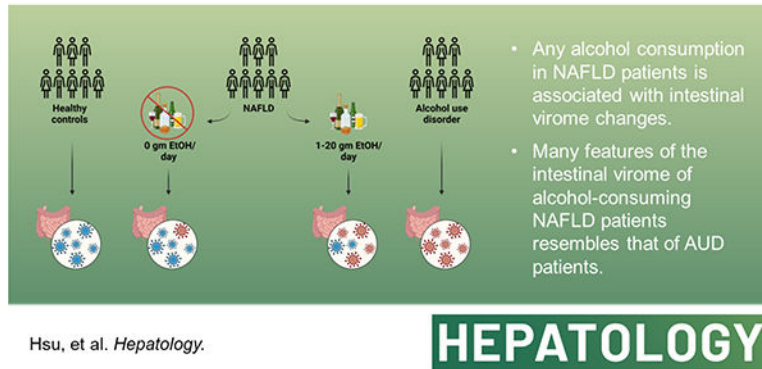
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alcohol consumption compared with no alcohol consumption. Conclusion: The intestinal virome of MAFLD patients who consume low to moderate amounts of alcohol are significantly different from those who do not, and many features of the intestinal virome of alcohol-consuming MAFLD patients resembles that of AUD patients.

Graphical Abstract

Does alcohol use in NAFLD patients affect their intestinal virome?



Keywords

Metabolic dysfunction-associated fatty liver disease (MAFLD); Nonalcoholic steatohepatitis (NASH); alcohol use disorder; alcohol-associated liver disease; microbiome

Introduction

Fatty liver disease, the most common chronic liver disease worldwide, can be caused by alcohol consumption or metabolic risk factors. Traditionally, patients are diagnosed with nonalcoholic fatty liver disease (NAFLD) if they have hepatic steatosis in the absence of significant alcohol use (limited to 20 grams of alcohol per day for women and 30 grams of alcohol per day for men) or other known causes of liver disease. However, due to the rising incidence of both obesity and alcohol use and therefore the increased likelihood of concurrence of these two disease etiologies, the new nomenclature metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed (1). MAFLD is diagnosed with hepatic steatosis in the presence of elevated BMI, type 2 diabetes mellitus (T2DM) or other metabolic risk factors, irrespective of the quantity of alcohol use.

Another somewhat controversial topic is the impact of low levels of alcohol intake on the development and progression of NAFLD. While some studies suggest an association of lower risk of development and progression of NAFLD with low to moderate alcohol consumption, others suggest increased risk for disease progression (2–6). Understanding the impact of alcohol use in NAFLD was demonstrated to be very important by a recent study where alcohol consumption in NAFLD patients measured by hair ethylglucuronide levels revealed repeated moderate to excessive alcohol consumption in 28.6% of NAFLD patients (7), suggesting that alcohol-associated liver disease may play a larger role than previously understood in the progression of liver disease in patients diagnosed with NAFLD.

The intestinal microbiome is known to play an important role in the progression of liver disease of all etiologies, and recent studies have found that progression of both alcohol-associated liver disease (ALD) and NAFLD are associated with significant changes in the intestinal virome (8, 9). However, while a few studies have investigated the effect of low-level alcohol consumption on the microbiomes of NAFLD patients (10, 11), none have investigated the effects on the intestinal virome. In this study, we characterize differences in the intestinal virome between alcohol use disorder (AUD) and NAFLD patients and explore the impact of low-moderate levels of alcohol use on the intestinal virome of MAFLD patients who were reclassified according to the proposed criteria.

Materials and Methods

Patient cohort

Our patient cohort and study design has been previously described in detail (9, 12). In brief, our cohort consisted of 21 control patients, 62 AUD patients, and 72 NAFLD patients. AUD patients, all heavy drinkers consuming over 60g of alcohol per day for more than 1 year, were prospectively enrolled at St. Luc University Hospital in Brussels, Belgium from April 2017 to January 2019, where they were admitted for a highly standardized and controlled 3-week detoxification and rehabilitation program, during which transient elastography was performed and a fasting blood sample and stool sample was collected on the day of admission and after two weeks of abstinence. Exclusion criteria included use of antibiotics, probiotics, or prebiotics during the two months preceding enrollment, use of immunosuppressive medications, diabetes, inflammatory bowel disease, known liver disease of any other etiology, or clinically significant cardio-vascular, pulmonary, or renal co-morbidities, and age < 18 years. NAFLD patients, diagnosed by the presence of steatosis in >5% of hepatocytes on liver biopsy or by clinical, laboratory, and imaging findings consistent with cirrhosis, were prospectively enrolled at the University Hospital of Cologne in Cologne, Germany from March 2015 to December 2018. Exclusion criteria included antibiotic use within 6 months prior to the study, known malignancy, pregnancy, and age < 18 years. These subjects were compared to healthy volunteers enrolled in Cologne, Germany (n = 5) and Brussels, Belgium (n = 16), matched for gender, age, and body mass index (BMI), who drank less than 20 g of alcohol per day. Data from AUD patients (8, 13) and NAFLD patients (9, 14) have been reported upon in prior studies.

Ethics

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's human research and ethical committee (Université Catholique de Louvain, Brussels, Belgium; B403201422657 and the local Ethics Committee at the University of Cologne, Germany; # 15–056), as previously described, and patients were enrolled after written informed consent was obtained. (9, 12, 15). We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria for reporting cohort studies.

Serum biomarkers

All blood samples were collected under fasting conditions. AUD patient blood samples were tested at the clinical laboratory associated with St. Luc University Hospital, Brussels, Belgium.

NAFLD patient blood samples were tested at the University Hospital of Cologne, Germany. Type 2 diabetes was defined as glycated hemoglobin A1c (HbA1c) of over 6.5% and/or fasting glucose level of over 126 mg/dL and/or use of antidiabetic medications.

Liver Stiffness and Steatosis Measurement

Vibration-controlled transient elastography (FibroScan, Echosens, Paris, France) was performed in fasting patients by experienced operators, blinded to all clinical patient data. At least 10 valid measurements were performed, and the median value of these measurements was reported in kilopascals. Patients were first scanned with the M probe, and if indicated by the equipment, patients were rescanned with the XL probe, in accordance with the manufacturer's protocol. Liver stiffness measurement cut-off of 7.5 kPa was used to discriminate mild fibrosis (stage F0–1) from advanced fibrosis (stage F2–4) (16) and controlled attenuation parameter cut-off of 248 dB/M was used for significant steatosis (17).

Virome preparation and metagenomic sequencing

Viral nucleic acids were extracted from fecal samples, reverse transcribed, and subjected to metagenomic sequencing using the Novel enrichment technique of VIROMES (NetoVIR) protocol with minor modifications as described previously (8, 9, 13, 18). Raw sequences are available for download in the NCBI Sequence Read Archive (SRA) associated with Bioproject PRJNA540738 and PRJNA786875. Corresponding biosample IDs to sample names for PRJNA786875 are listed in Supplementary Table 1.

Virome Analysis

Raw sequence reads were processed as previously described (9, 13). Briefly, raw sequence reads were deduplicated using Clumpify (<https://sourceforge.net/projects/bbmap/>) followed by trimming and filtering for low-quality and contaminating human reads using Kneaddata (19) with the GRCh38_v25 human genome reference. Profiling the composition of the microbial communities of enriched viromes was accomplished using Metaphlan3 (20). Specifically, filtered reads from each sample were used as input for the following command: "metaphlan R1.fastq, R2.fastq --bowtie2out ./sample_name.bowtie2.bz2 --nproc 5 --input_type fastq --add_viruses --unknown_estimation". All output abundance profiles were combined using the command: "merge_metaphlan_tables.py *_profiled_metagenome.txt > merged_abundance_metaphlan_table.txt". The viral component was extracted from the merged abundance table and relative abundance of each virus in relation to all viruses extracted per sample was calculated and imported into R (R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis and generation of plots. Eight samples were sequenced twice, and the resulting read counts were averaged (Supplementary Table 1).

Statistical Analysis

For parametric data (e.g., serum markers), the Student's t-test was used for comparison between two groups and the one-way analysis of variance with Tukey's post-hoc test was used for three or more groups. Results were expressed as mean and standard deviation for each continuous outcome, if not stated otherwise. For non-parametric data (e.g., virome data), the Mann–Whitney U test/Wilcoxon rank-sum test was used for comparison between two groups and the Kruskal–Wallis test with Dunn's post-hoc test was used for three or more groups. All statistical tests were two-sided. Relative abundances for further analyses were calculated at the species level for single phages. Principle coordinate analysis (PCoA) using Chi-square distance matrices was used to identify differences in the relative abundance of virus species. P values were determined by permutational multivariate analysis of variance (PERMANOVA) while adjusting for potentially confounding factors. Linear discriminant effect-size analysis (LEfSe) was performed to determine the features most likely to account for differences between groups (21). A P value equal to or less than 0.05 was considered statistically significant. Statistical analysis was performed by using R statistical software (R version 4.0.3; R Foundation for Statistical Computing).

Results

Differences in fecal virome composition between NAFLD and AUD patients

Control subjects from University of Cologne and St. Luc University Hospital were compared and there were no significant differences in their demographic or laboratory parameters, including age, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyltransferase (GGT), bilirubin, or albumin, other than body mass index (BMI) (Supplementary Table 2). The mean BMI was within normal range for both groups. The intestinal viromes of control subjects from the two study sites were also compared and found to have no significant differences (Supplementary Figure 1).

Compared with control and AUD patients, NAFLD patients were older and had higher BMIs. Compared with AUD patients, control and NAFLD patients had significantly lower AST, ALT, and GGT. Control subjects had significantly lower AP than AUD or NAFLD patients (Table 1). The intestinal viromes of 62 AUD patients (at both active alcohol use and abstinent time points) were compared with those of 72 NAFLD patients and found to have significant differences by PCoA analysis (Figure 1A). Linear discriminant effect-size analysis (Lefse) was applied to identify features most likely to account for differences between the two groups. This revealed one *Bacteroides* phage more abundant in the NAFLD population and two *Lactococcus* phages more abundant in the AUD population (Figure 1B–E). The two AUD patients with the highest levels of *Lactococcus phage P335* were also noted to have higher AST and ALT levels (Supplementary Figure 2).

Given differences in the gender composition of the NAFLD and AUD cohorts, we compared the viromes of the two groups between males and females and found no significant differences in the intestinal virome between the two genders in either group (Supplementary Figure 3). Next, we performed nearest neighbor propensity score matching for age and gender and observed improved balance of both age and gender in the matched cohorts

(Supplementary Figure 4A–C). Significant differences in the intestinal viromes of NAFLD and AUD patients persisted in the matched cohorts (Supplementary Figure 4D).

Alcohol use in MAFLD patients is associated with fecal virome differences

Using our cohort of NAFLD patients, 69 patients (95.8%) were reclassified with the diagnosis of MAFLD using published MAFLD criteria (1). Of these patients, 33 reported no alcohol use and 36 reported some alcohol use (limited to 20 grams of alcohol per day for both genders). MAFLD patients who did not consume alcohol were significantly more obese than those who did consume alcohol but were comparable in other demographic and laboratory parameters (Table 2). The fecal viromes of MAFLD patients who consume alcohol was significantly different from those who do not consume alcohol (Figure 2A). The viromes of MAFLD patients who do not consume alcohol appeared more similar to the control population, while the viromes of MAFLD patients who consume alcohol appeared more similar to AUD patients. Several species of *Lactococcus* phages were the most discriminating features of MAFLD patients who consumed alcohol and AUD patients by LEfSe analysis (Figure 2B). The relative abundance of these *Lactococcus* phages were significantly higher in MAFLD patients who consumed alcohol and similar to AUD patients, as compared to control subjects and MAFLD patients who do not consume alcohol (Figure 2C, D, Supplementary Figure 5). We performed the same analyses using the age/gender matched cohort derived above and found very similar results (Supplementary Figure 6 A–D).

Next, Random Forest feature selection was used to analyze the 117 patients in our cohorts with steatosis (CAP cut-off of 248 dB/M) or advanced fibrosis (including 46 AUD patients, 34 MAFLD patients who did not consume alcohol, and 37 MAFLD who consumed alcohol) to extract features most important for discriminating between any alcohol consumption and no consumption. Of the top 15 phages identified as important for differentiating these two populations, 9 were *Lactococcus* phages (Figure 2E). We next performed multivariate logistic regression analyses using the top 7 *Lactococcus* phages identified by Random Forest feature selection (*Lactococcus* phage jm2, *Lactococcus* phage jm3, *Lactococcus* phage bIL67, *Lactococcus* phage ul36, *Lactococcus* phage Tuc2009, *Lactococcus* phage 340, *Lactococcus* phage phi7) to predict alcohol use in patients with steatosis or advanced fibrosis and compared this with the predictive power of the ALD/NAFLD Index, a scoring system using mean corpuscular volume, AST/ALT ratio, BMI, and gender to distinguish ALD from NAFLD (22). While the ALD/NAFLD Index had an AUC of 0.56 (95% CI: 0.4237–0.6984) for predicting alcohol use in patients with steatosis or advanced liver disease, the top 7 *Lactococcus* phages had an AUC of 0.77 (95% CI: 0.6787–0.8624) (Figure 2F). Together this data suggests that even low to moderate amounts of alcohol use (less than 20 grams/day) is associated with significant differences in virome composition.

Alcohol use is associated with differences in intestinal virome in patients with advanced fibrosis

Of the 43 patients in our cohort with advanced fibrosis (F2–4), significant differences in the intestinal virome were seen between MAFLD patients who consumed alcohol, those who did not, and AUD patients (Figure 3A). The relative abundance of *Lactococcus* phage

jm2 was significantly higher in MAFLD patients who consumed alcohol and AUD patients with mild fibrosis than in control subjects, and the trend towards higher abundance in patients who used alcohol persists in advanced fibrosis (Figure 3B). In the advanced fibrosis population, the relative abundance of *Lactococcus* phage jm3 was significantly higher in AUD patients than in MAFLD patients who did not consume alcohol (Figure 3C). Similarly, while there was a trend towards increased relative abundance of *Lactococcus* phage bIL67 in alcohol users in the mild fibrosis patient population, there was a significant increase in MAFLD patients with advanced fibrosis who used alcohol compared to those who did not (Figure 3D).

Differences in intestinal virome between low and moderate alcohol use

MAFLD patients were further classified as no alcohol use (n = 33), low alcohol use (n = 25) defined as less than 10 grams of alcohol per day, and moderate alcohol use (n = 11) defined as less than 20 grams of alcohol per day, regardless of age. The average alcohol consumed was 3.3 grams/day for the low alcohol-consumption group and 11.1 grams/day for the moderate alcohol-consumption group (Table 3). The only other significant difference was an elevated bilirubin level in moderate alcohol-consumers compared to no and low alcohol-consumers (p = 0.008). When these groups were compared with control subjects and AUD patients, their fecal viromes were significantly different (Figure 4A). Additionally, *Lactococcus* phage bIL67 and *Lactococcus* phage jm2 were again the most discriminating factors for low versus moderate alcohol-consumers (Figure 4B). The relative abundance of *Lactococcus* phage bIL67 was significantly higher in moderate alcohol-consuming MAFLD patients compared to no and low alcohol-consuming MAFLD patients (Figure 4C), whereas the relative abundance of *Lactococcus* phage jm2 was significantly higher in low alcohol-consuming MAFLD patients compared to no and moderate alcohol-consuming MAFLD patients (Figure 4D). Additionally, the diversity of the intestinal virome as quantified by the Shannon index was significantly different between control subjects, MAFLD patients who did not consume alcohol, those who did, and AUD patients, with significant differences specifically between those who did not consume alcohol and those who did (Figure 4E). These data again highlight differences in the intestinal virome with even small increases in alcohol use.

Discussion

In this study, we compare the intestinal viromes of NAFLD/MAFLD and AUD patients with a spectrum of liver disease stages. Though many studies investigate pathological alcohol intake and NAFLD separately, few studies specifically focus on the role of modest alcohol intake in NAFLD patients and the findings from these studies have been varied (2, 3, 6). Clearly, there is a need for additional studies evaluating the effect of modest alcohol use among NAFLD patients.

We demonstrate not only that there are significant differences in the intestinal viromes of NAFLD and AUD patients, but that alcohol use in NAFLD patients reclassified with MAFLD accounted for significant differences in the intestinal viromes. In fact, the relative abundance of several *Lactococcus* phages was more similar between AUD patients and

alcohol-consuming MAFLD patients than non-alcohol-consuming MAFLD patients and control subjects, and multivariate modeling using top distinguishing *Lactococcus* phages could better predict alcohol use in the MAFLD population than existing validated models such as the ALD/NAFLD Index. There were even significant differences in the viral composition between MAFLD patient with low (average 3.3 grams/day) and moderate (average 11.1 grams/day) alcohol consumption. One limitation of our study is the lack of availability of precise alcohol consumption data for the healthy control subjects. Though all consumed less than 20 grams/day of ethanol, more granular differences in alcohol consumption may explain some of the variability seen in the intestinal viromes of the healthy controls.

Alcohol use disorder patients have previously been shown to harbor higher abundance of the bacterial genera *Lactococcus* in their gut microbiota (23), and in patients with alcohol-associated hepatitis, those with the most severe steatosis had significantly increased fecal abundance of *Lactococcus* (24). Hence, the finding of increased intestinal abundance of *Lactococcus* phages with alcohol use seen in our study may reflect the phage-bacteria relationship in the setting of increased *Lactococcus* bacteria with alcohol use. Increased intestinal abundance of *Lactococcus* phages has also been associated with more progressed liver disease (8), though it is unclear whether the abundance of *Lactococcus* phages directly influences liver disease progression or is a result.

Further, the viral diversity of the alcohol-consuming MAFLD population was similar to the AUD population and significantly higher than the non-alcohol-consuming MAFLD population. In the context of previous studies showing increased intestinal viral diversity in ALD and decreased intestinal viral diversity in NAFLD (9, 13), this data suggests that any alcohol consumption is associated with changes in the intestinal viral composition of patients with a NAFLD diagnosis. NAFLD is a diagnosis given in the absence of significant alcohol use and all NAFLD patients in our study cohort reported alcohol use of less than 20 grams/day irrespective of gender. However, our results suggest that even low to moderate amounts of alcohol use is associated with changes in intestinal virome composition, which raises the question of whether any alcohol use is appropriate in NAFLD patients and whether the diagnosis of exclusion unnecessarily creates dichotomized, mutually exclusive diagnoses when the pathophysiology is more likely to resemble a spectrum.

Overall, our study shows that any alcohol consumption is associated with changes in the intestinal virome in NAFLD patients and these changes reveal an intestinal virome that more closely resembles that of AUD patients. These results reinforce the importance of careful evaluation for alcohol consumption in patients with presumed NAFLD and emphasize the role even a modest amount of alcohol may play on changes in the intestinal virome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations:

LT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
ALD	alcohol-associated liver disease
AUD	alcohol use disorder
BMI	body mass index
CAP	controlled attenuation parameter
GGT	gamma-glutamyltransferase
INR	international normalized ratio
LEfSe	linear discriminant effect-size analysis
LSM	liver stiffness measurement
MAFLD	metabolic dysfunction-associated fatty liver disease
NAFLD	non-alcoholic fatty liver disease
PCoA	Principal coordinate analyses
ROC	Receiver operating characteristic

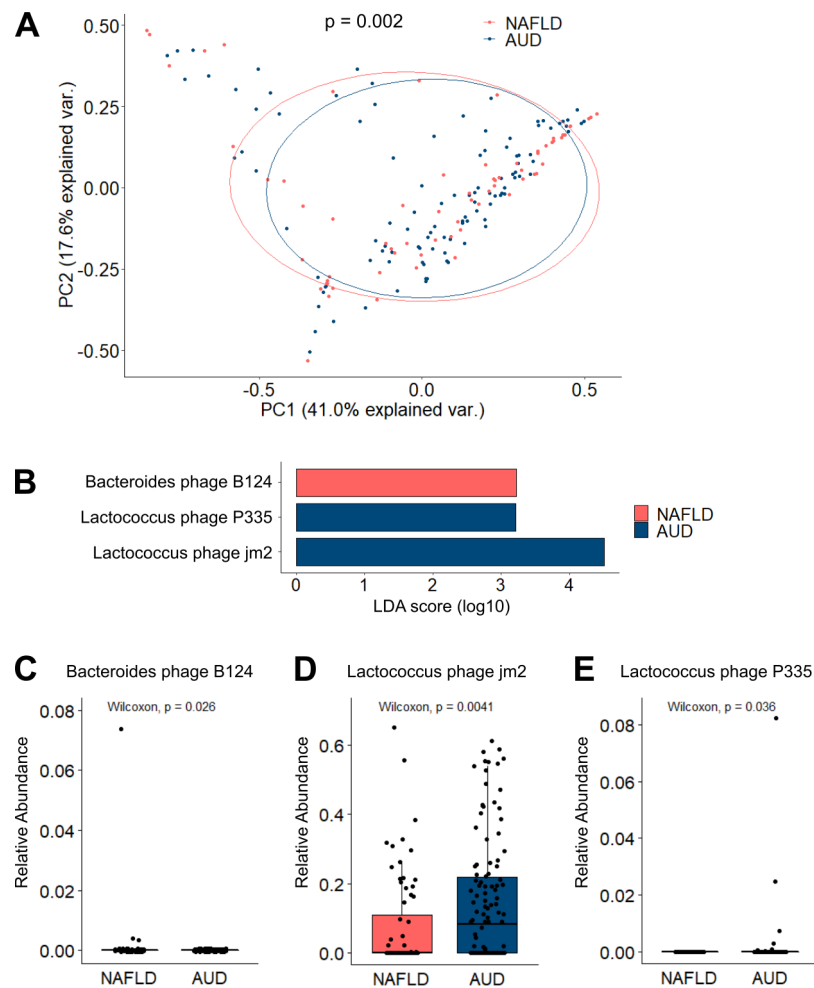
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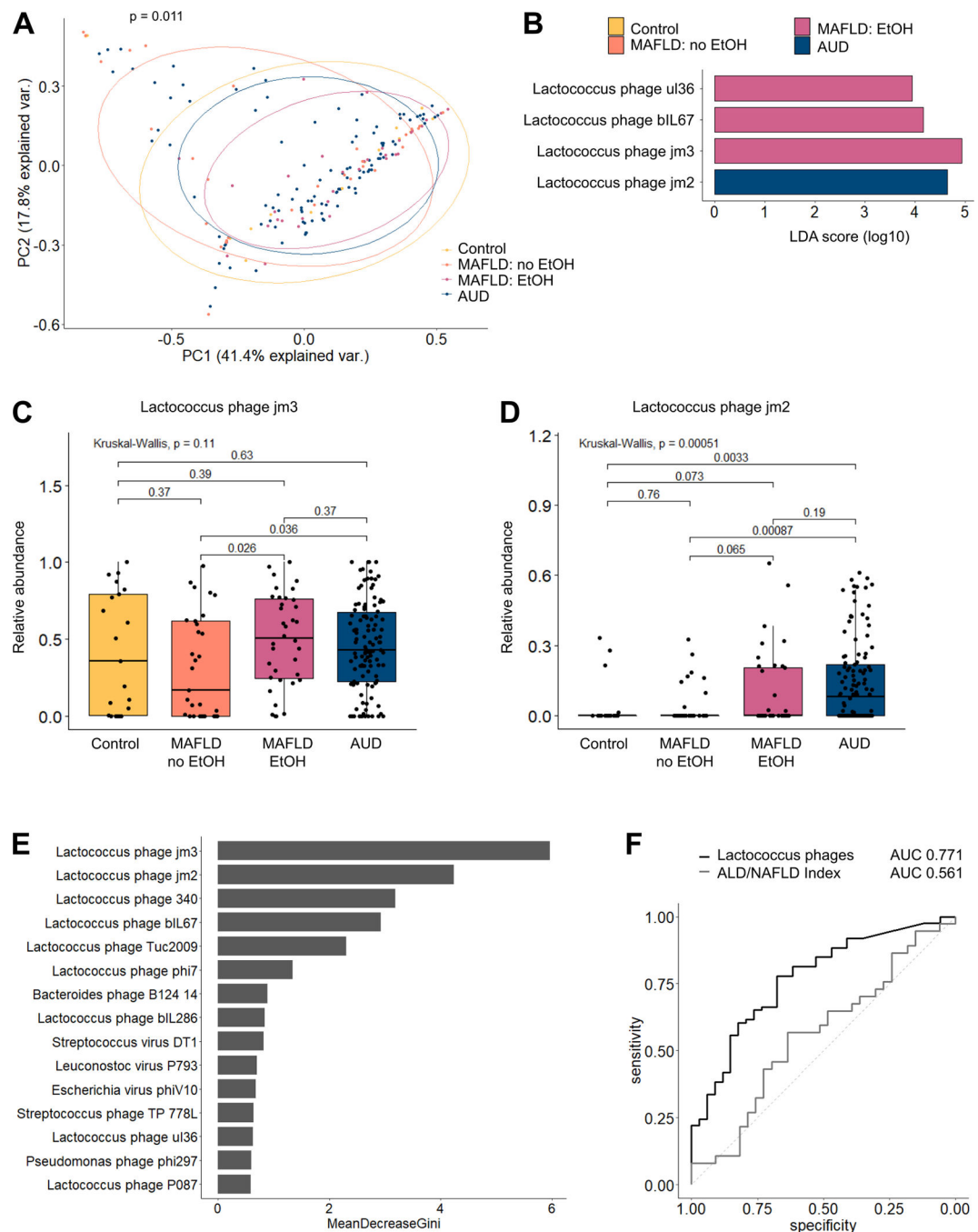
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**Figure 1:**

The intestinal virome is different between NAFLD and AUD patients. **(A)** Principal coordinate analysis (PCoA) of intestinal viruses in all NAFLD and all AUD patient samples. **(B)** Linear discriminant analysis (LDA) of bacteriophage species in NAFLD versus AUD patients. **(C-E)** Relative abundance of **(C)** Bacteroides phage B124, **(D)** Lactococcus phage jm2, and **(E)** Lactococcus phage P335.

**Figure 2:**

In MAFLD patients, any alcohol use is associated with significant differences in the intestinal virome compared with complete abstinence. **(A)** PCoA of intestinal viruses in control subjects, MAFLD patients who did not consume any alcohol, those who consumed some alcohol, and AUD patients. **(B)** LDA of bacteriophage species in control subjects, MAFLD patients who did not consume any alcohol, those who consumed some alcohol, and AUD patients. **(C-D)** Relative abundance of **(C)** Lactococcus phage jm3 and **(D)** Lactococcus phage jm2. **(E)** Random forest feature selection using relative abundance of

viral species to discriminate between non-alcohol users and alcohol users. **(F)** Receiver operating characteristic (ROC) curves were performed based on multivariate models to predict the presence of alcohol use. The top seven most important Lactococcus phages identified by random forest feature selection were included in the multivariate phage model and compared to a model using the ALD/NAFLD index.

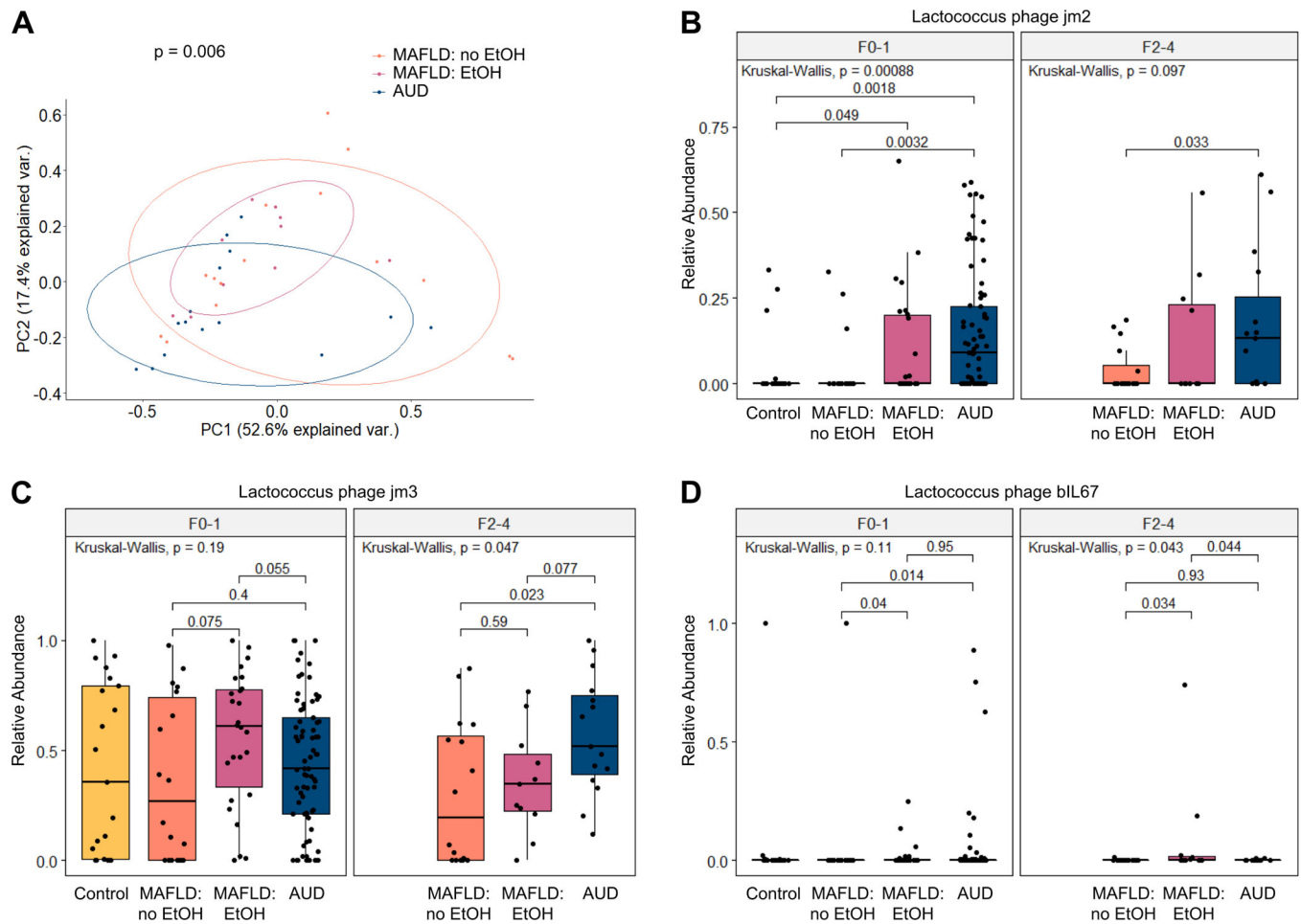
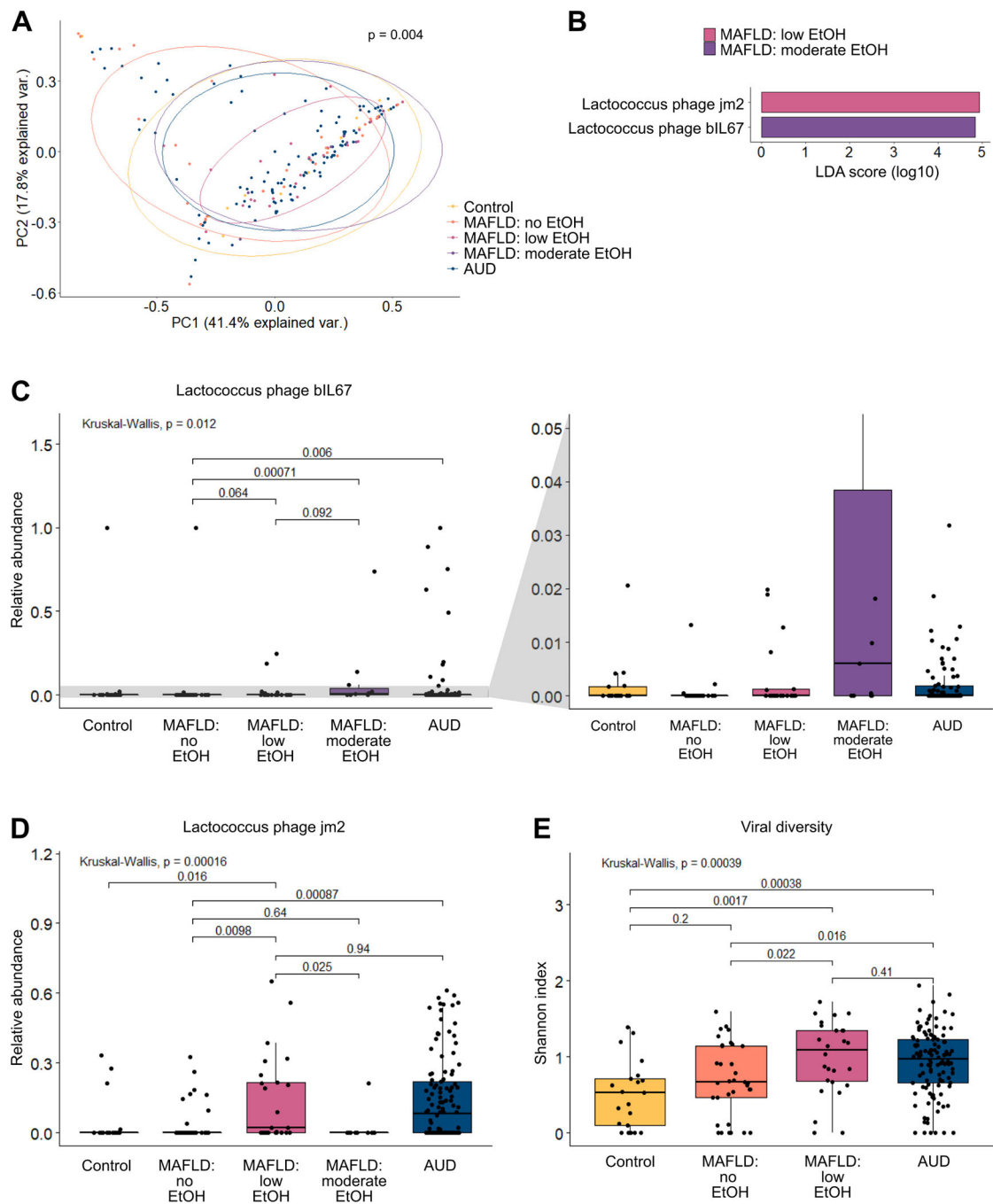


Figure 3:

In patients with advanced fibrosis, significant differences in the intestinal virome are still seen between completely abstinent MAFLD patients and those who consume alcohol. **(A)** PCoA of intestinal viruses in patients with advanced fibrosis (F2–4) and either MAFLD (no alcohol use), MAFLD (any alcohol use), or AUD. **(B–D)** Relative abundance of **(B)** Lactococcus phage jm2, **(C)** Lactococcus phage jm3, and **(E)** Lactococcus phage bIL67, faceted by severity of hepatic fibrosis stage.

**Figure 4:**

There are significant differences in the intestinal virome of MAFLD patients with mild alcohol consumption (<10 g/day) vs moderate alcohol consumption (10+ g/day) vs no consumption, compared to AUD. **(A)** PCoA of intestinal viruses in control subjects, MAFLD patients who did not consume any alcohol, those with low alcohol consumptions, those with moderate alcohol consumption, and AUD patients. **(B)** LDA of bacteriophage species in control subjects, MAFLD patients who did not consume any alcohol, those with low alcohol consumptions, those with moderate alcohol consumption, and AUD patients.

(C-D) Relative abundance of **(C)** Lactococcus phage bIL67 and **(D)** Lactococcus phage jm2.
(E) Viral diversity based on the Shannon Index.

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Table 1.

Demographic and laboratory parameters of the study population.

Characteristics	Control (n = 21)	Alcohol use disorder (n = 62)	NAFLD (n = 72)	P value
Age (years), n = 155	43.3 ± 12.8	44.4 ± 11.9	53.0 ± 13.4	<0.001
Sex (male), n (%), n = 155	15 (71.4%)	44 (71.0%)	35 (48.6%)	0.017
BMI (kg/m ²), n = 155	23.1 ± 3.6	24.3 ± 3.8	31.2 ± 5.7	<0.001
AST (IU/L), n = 146	19.7 ± 5.2	67.5 ± 64.7	42.1 ± 25.2	<0.001
ALT (IU/L), n = 146	12.6 ± 4.1	53.3 ± 42.3	57.4 ± 38.4	<0.001
GGT (IU/L), n = 145	21.9 ± 10.6	208.5 ± 291.9	112.4 ± 123.7	0.003
AP (IU/L), n = 144	50.4 ± 18.6	79.1 ± 33.6	81.0 ± 25.8	0.002
Bilirubin (mg/dL), n = 145	0.42 ± 0.52	0.55 ± 0.37	0.65 ± 0.46	0.148
Albumin (g/dL), n = 142	4.5 ± 0.2	4.6 ± 0.4	4.4 ± 0.3	0.001
INR, n = 135	1.0 ± 0	0.98 ± 0.11	1.03 ± 0.2	0.324
Creatinine (mg/dL), n = 146	0.91 ± 0.20	0.80 ± 0.14	0.86 ± 0.20	0.052
Platelets (10 ⁹ /L), n = 137	255 ± 55	228 ± 79	223 ± 70	0.632
CAP, n = 90	233.6 ± 56.9	285.1 ± 57.9	289.0 ± 49.1	0.124
LSM (kPa), n = 136	4.56 ± 1.02	7.67 ± 9.71	9.28 ± 7.43	0.329
*Post-hoc P values:				
Characteristics	Control vs AUD	Control vs NAFLD	AUD vs NAFLD	
Age	0.944	0.008	<0.001	
Sex	0.968	0.130	0.026	
BMI	0.594	<0.001	<0.001	
AST	0.002	0.239	0.005	
ALT	0.002	0.812	<0.001	
GGT	0.011	0.324	0.025	
AP	0.004	0.002	0.924	
Albumin	0.271	0.829	<0.001	

Values presented are mean ± standard deviation. The number of subjects for which data were available is indicated in the first column.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; INR, international normalized ratio; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Table 2.

Demographic and laboratory parameters of the MAFLD study population.

Characteristics	MAFLD: All patients (n = 69)	MAFLD: No EtOH (n = 33)	MAFLD: EtOH (n = 36)	P value
Age (years)	53.0 ± 13.3	53.0 ± 12.4	53.0 ± 14.	0.886
Sex (male), n (%)	34 (49.3%)	15 (45.5%)	19 (52.8%)	0.358
BMI (kg/m ²)	31.6 ± 5.5	33.0 ± 6.6	30.2 ± 4.0	0.028
AST (IU/L)	41.9 ± 25.6	41.5 ± 21.9	42.3 ± 28.7	0.310
ALT (IU/L)	58.0 ± 39.0	54.2 ± 31.9	61.4 ± 44.6	0.164
GGT (IU/L)	111.3 ± 125.1	129.3 ± 162.5	95.2 ± 77.4	0.514
AP (IU/L)	80.4 ± 26.1	84.2 ± 26.6	77.0 ± 25.5	0.794
Bilirubin (mg/dL)	0.6 ± 0.4	0.56 ± 0.33	0.68 ± 0.44	0.019
Albumin (g/dL)	43.9 ± 3.1	43.2 ± 2.7	44.5 ± 3.3	0.145
INR	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	0.802
Creatinine (mg/dL)	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.719
Platelets (10 ⁹ /L)	224.5 ± 69.1	234.8 ± 76.6	215.3 ± 61.5	0.078
CAP	291.5 ± 48.8	287 ± 33.7	295.3 ± 59.8	0.508
LSM (kPa)	8.8 ± 6.0	10.0 ± 6.6	7.9 ± 5.4	0.416

Values presented are mean ± standard deviation. The number of subjects for which data were available is indicated in the first column.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; INR, international normalized ratio; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

Table 3.

Demographic and laboratory parameters of the MAFLD population stratified by no, low, and moderate alcohol use.

Characteristics	MAFLD: No EtOH (n = 33)	MAFLD: Low EtOH (n = 25)	MAFLD: Moderate EtOH (n = 11)	P value
Age (years)	53.0 ± 12.4	52.6 ± 15.1	54.0 ± 13.0	0.963
Sex (male), n (%)	15 (45.5%)	12 (48.0%)	7 (63.6%)	0.584
Alcohol use (g/day)	0 ± 0	3.3 ± 2.3	11.1 ± 1.9	<0.001
BMI (kg/m ²)	33.0 ± 6.6	30.7 ± 4.3	29.2 ± 3.1	0.088
AST (IU/L)	41.5 ± 21.9	36.6 ± 14.2	55.1 ± 46.3	0.134
ALT (IU/L)	54.2 ± 31.9	54.7 ± 31.4	76.8 ± 64.9	0.220
GGT (IU/L)	129.3 ± 162.5	84.9 ± 62.0	118.6 ± 104.3	0.409
AP (IU/L)	84.2 ± 26.6	72.3 ± 18.7	87.6 ± 35.4	0.139
Bilirubin (mg/dL)	0.6 ± 0.3	0.6 ± 0.3	1.0 ± 0.6	0.008 *
Albumin (g/dL)	43.2 ± 2.7	44.5 ± 2.8	44.4 ± 4.5	0.249
INR	1.0 ± 0.3	1.0 ± 0.1	1.1 ± 0.2	0.599
Creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.596
Platelets (10 ⁹ /L)	234.8 ± 76.6	227.5 ± 57.0	187.6 ± 65.0	0.144
CAP	287 ± 33.7	288.1 ± 67.7	309.5 ± 45.0	0.735
LSM (kPa)	10.0 ± 6.6	7.2 ± 3.9	9.4 ± 7.8	0.201

Values presented are mean ± standard deviation. The number of subjects for which data were available is indicated in the first column.

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; INR, international normalized ratio; CAP, controlled attenuation parameter; LSM, liver stiffness measurement.

* Post-hoc P values for Bilirubin: No EtOH versus Mild EtOH, P = 0.995; No EtOH versus Moderate EtOH, P = 0.011; Mild EtOH versus Moderate EtOH, P = 0.011.