EARLY SHIFT IN GUT MYCOBIOME EXACERBATES ALCOHOL-RELATED LIVER DISEASE PROGRESSION

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INTRODUCTION

Despite its minor but crucial representation in gut, the role of gut fungal community remains underexplored particularly in alcohol-related liver disease (ALD). Here, we meticulously assessed the pathophysiological role of gut mycobiome, in ALD severity progression.

METHOD

Feces collected from 68 ALD and 21 healthy individuals for Internal Transcribed Spacer 2 (ITS2) based comparative mycobiome assessment, followed a by gut fungal and bacterial suppressed NIAAA alcoholic mice model to explain the role of gut fungus in ALD.

RESULTS

MELD score-based classification showed a decisive MELD score severity-associated shift in fecal fungal ecology in ALD, while target read counts followed the MELD score severity pattern, but no changes were observed in operational taxonomic units (OTUs) or in alpha diversity indexes. Significant alterations occurred in phylum's (*Basidiomycota*, *Plantae_p*, and *Mucoromycota*) and in genus (*Saccharomyces*, *Kazachstania*, *Geotrichum*, and *Trichosporon*). Among 105 common fungal species, 3 (*S. cerevisiae*, *S. kudriavzevii*, and *A. penicillioides*) exhibited ALD-associated rises. Fungus species including *A. penicillioides* (AUC: 0.82) and *P. polonicum* (AUC: 0.80) served as biomarkers, and their combined ability improved the AUC upto 0.87. Interestingly, alcohol-producing fungal species showed significant increments in ALD patients, with their highest abundance observed at the early stage of ALD, and inversely correlated with MELD scores. Furthermore, antibiotic treatment (ABX), in NIAAA murine model showed significantly higher mortality, decreased body weight and liver weight compared to the alcoholic control and antifungal treatment groups, ABX also presented elevated serum AST, cholesterol, and total bilirubin levels, and upregulated the expression of alcohol dehydrogenase (***ADH1***) and Cytochrome P450 IIE1 (***CYP2E1***) genes involved in oxidative alcohol metabolism in the liver. Total fecal fungal load increased significantly in ABX treatment group compared to other groups. Relative expression of liver fibrotic markers genes such ***TGF-β***, ***TNF-α***, ***Col1a1*** and ***ACTA2*** showed significant increment in ABX group.

RESULTS

Clinical Parameters	Healthy Control (Total sample n=21)	Alcoholic Liver Diseased Group		
		Hepatitis (Total sample n=31)	Cirrhosis (Total sample n=65) (n=47, 72%)	
			Alive	Dead
PT (n)	7 (33%)	21 (68%)	31	16
Female	5 (71%)	3 (14%)	10(32%)	2(13%)
Age	78.6 ± 6.3	55.20 ± 8.1	54.13 ± 11.0	60.19 ± 8.7
BMI	21.5 ± 3.5	23.08 ± 3.4	23.79 ± 2.7	24.30 ± 3.0
Alcohol Consumption	No	Yes	Yes	Yes
Liver Conditions	No	Yes	Yes	Yes
Ascites	No	No	Yes (n=31)	Yes (n=16)
Bleeding	No	No	Yes (n=6)	Yes (n=2)
Encephalopathy	No	Yes (n=21)	Yes (n=31)	Yes (n=16)
Fungal infection	No	No	No	No
Antifungal Treatment	No	No	No	No
Cholesterol (mg/dL)	121.4 ± 34.4	144.80 ± 40.2	148.63 ± 42.9	99 ± 41.0***
HDL (mg/dL)	38.3 ± 9.2	46.29 ± 28.3	39.50 ± 15.2	25.87 ± 11.3**
TG (mg/dL)	98.1 ± 27.0	134.99 ± 93.9	160.34 ± 80.9	86.31 ± 35.6**
AST (U/L)	28.1 ± 5.4	54.40 ± 28.5**	63.68 ± 31.1**	57.50 ± 24.**
ALT (U/L)	24 ± 10	46.93 ± 40.2	40.86 ± 20.5*	24.06 ± 12.3*
Creatinine (mg/dL)	0.8 ± 0.1	0.85 ± 0.1	0.88 ± 0.4	0.83 ± 0.4
GGT (U/L)	29.4 ± 9.1	80.29 ± 52.6*	180 ± 157.1*	104.19 ± 145.0
Bilirubin (mg/dL)	0.8 ± 0.3	1.29 ± 1.0	2.36 ± 2.4*	5.94 ± 6.1***
Albumin	4.1 ± 0.5	3.69 ± 0.5	3.65 ± 0.7	3.11 ± 0.4***
Sodium (mEq/L)	141.9 ± 1.9	140.8 ± 2.9	136.68 ± 5.5*	136.31 ± 3.4*
Platelet (x10^9/L)	216.1 ± 47.4	227.21 ± 144.1	152.50 ± 110.5	73.19 ± 37.5**
PT (sec)	12.7 ± 1.2	12.53 ± 3.2	14.45 ± 2.9	20.12 ± 4.4***
INR	1.2 ± 0.1	1.08 ± 0.1	1.33 ± 0.2	1.83 ± 0.4***
MELD Score	8.1 ± 0.9	8.80 ± 2.6	12.68 ± 5.2*	19.25 ± 4.9***
MELD Na (UNOS version)	8.1 ± 0.9	8.93 ± 2.9	13.90 ± 6.8*	20.38 ± 5.7***
MELD Score 3.0	8.4 ± 1.5	9 ± 2.9	14.42 ± 6.7*	20.69 ± 5.1***

Table 1: Clinical parameters of participants included in the clinical trial.

Fig.1

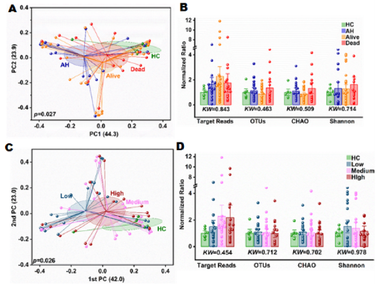


Figure 1. Gut fungal ecology in ALD changed with MELD score. Fungal community in gut increased in as MELD score increased. (A, C) Beta-diversity analyses reveal distinct fungal community structures across conventional disease groupings and when stratified by MELD score categories, respectively. (B, D) Alpha diversity indices (e.g., richness, Shannon diversity) demonstrate increased fungal diversity within samples as MELD score increases, indicating progressive fungal dysbiosis correlating with ALD severity.

Fig.2

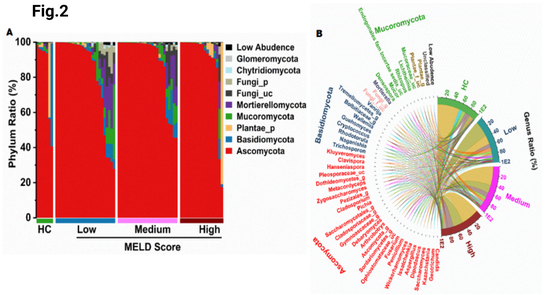


Figure 2. MELD-dependent shifts in gut fungal composition at phylum and genus levels in ALD. (A) Phylum-level relative abundance across MELD categories. (B) Genus-level relative abundance across MELD categories

Fig.3

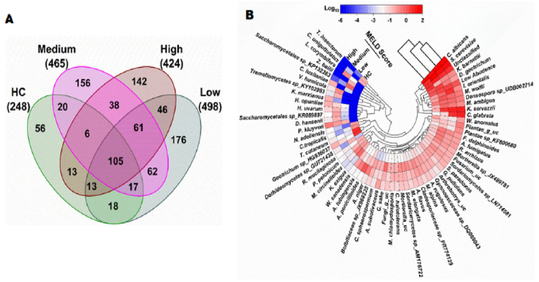
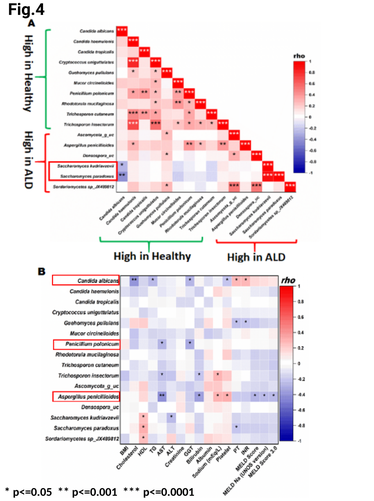


Figure 3. Species-level alterations in the gut mycobiome across MELD categories in ALD. (A) Species richness (number of observed fungal species) stratified by MELD category. (B) Relative abundance of the most abundant species differentiating MELD categories

Fig.4



* p<=0.05 ** p<=0.001 *** p<=0.0001

Figure 4. Correlation between of gut fungal species and clinical markers in ALD. (A) Pairwise species–species correlation taxa enriched in ALD with enriched in healthy controls, (B) Correlations between ALD-enriched species and clinical markers

Fig.5

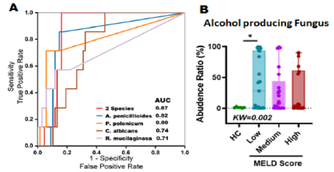


Figure 5. Biomarker potential of fungal species and early enrichment of ethanol-producing taxa in ALD. (A) Biomarker ability, (B) Relative abundance of ethanol-producing fungi across disease stages

Fig.6

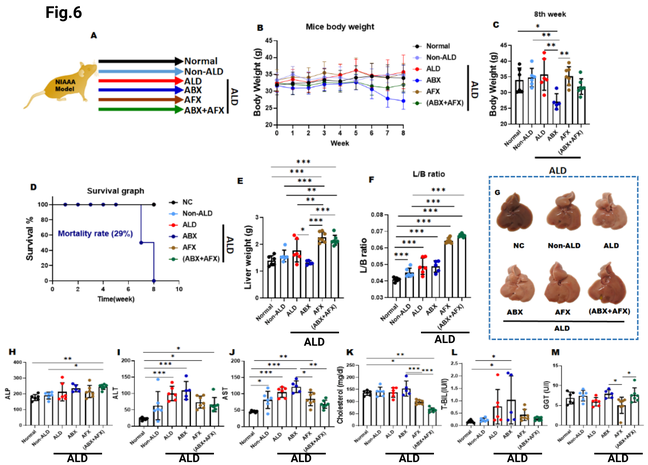


Figure 6. Expansion of gut fungi exacerbates alcohol-induced liver injury in mice. (A) Schematic of the murine study and fungal manipulation. (B,C) Body-weight trajectory and endpoint body weight, (D) Survival (mortality rate), (E) Absolute liver weight, (F) Liver-to-body weight ratio (L/B). (G) Representative gross liver images. (H–M) Serum biochemistry

Fig.7

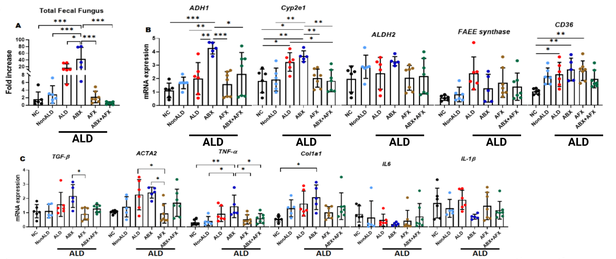


Figure 7. Gut fungal expansion exacerbates alcohol-related liver injury by amplifying oxidative pathways. (A) Fold change in intestinal fungal burden, (B) Hepatic expression of oxidative and non-oxidative ethanol-metabolism pathways, (C) Liver injury related molecular markers.

CONCLUSIONS

Chronic alcohol abuse significantly altered the gut fungal composition at early stage of the ALD and potentially exacerbating ALD severity.

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