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Gender-Stratified Gut Dysbiosis Patterns in HCC Patients: A Cross-Sectional Microbiome Study

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Abstract

Background: Hepatocellular carcinoma (HCC) exhibits significant sex disparities, with males demonstrating a higher incidence. While hepatitis C virus (HCV) drives hepatocarcinogenesis and the gut microbiome influences liver disease progression, Sex-specific microbiome signatures in HCC remain underexplored.

Methods: This cross-sectional study analyzed 36 participants stratified by sex and disease status: male controls (MC, n=9), male with HCC (MHCC, n=9), female controls (FC, n=9), and female with HCC (FHCC, n=9). Stool samples underwent 16S rRNA sequencing (V3-V4 regions).

Results: Profound sex-specific dysbiosis emerged: MHCC patients showed significant enrichment of pro-inflammatory *Proteobacteria* (*Succinivibrio*), correlating with coagulopathy (INR: r= 0.62) and thrombocytopenia (r= –0.61), while FHCC exhibited depletion of commensal *Prevotella_9* and enrichment of estrogen-associated *Eggerthella*, linked to hyperbilirubinemia (r= 0.54) and extreme AFP elevation (r= 0.49). Alpha diversity loss was universal but more severe in males (Shannon index, $p$adj= 0.008). Machine learning identified *Lachnospiraceae_ND3007_group* (male AUC = 0.81) and *Eggerthella* (female) as top discriminators.

Conclusion: Sex-stratified dysbiosis patterns reveal distinct HCC pathogenesis, inflammatory (*Proteobacteria*-driven) in males and metabolic (*Eggerthella*-mediated) in females. These signatures provide novel biomarkers for personalized risk stratification in HCV-associated HCC.

Keywords: Hepatocellular carcinoma, gut microbiome, Sex disparities, hepatitis C virus, microbial biomarkers, dysbiosis

1. Introduction

Hepatocellular carcinoma (HCC) represents the fourth leading cause of cancer-related mortality worldwide, characterized by aggressive progression and limited therapeutic options in advanced stages (Osama et al., 2025; Singh et al., 2025). This malignancy arises predominantly in cirrhotic livers, with major risk factors including chronic viral hepatitis (HBV/HCV), alcohol abuse, and metabolic dysfunction-associated steatohepatitis (MASH) (Llovet et al., 2021). Notably, HCC exhibits marked sex disparities, with males facing 2-3 times higher incidence and mortality rates compared to females, a divergence attributed to

hormonal influences, differential risk factor exposure, and sex-specific immune responses (Petrick et al., 2020). The five-year survival rate remains dismal at 18%, underscoring the urgent need for early detection biomarkers and mechanistic insights into sex-biased pathogenesis (Sung et al., 2021).

Chronic hepatitis C virus (HCV) infection drives hepatocarcinogenesis through direct viral oncogenic effects and chronic inflammation-mediated liver injury (Ringelhan et al., 2017). The virus promotes genomic instability via core protein interactions with p53 and Wnt/β-catenin pathways while inducing oxidative stress and mitochondrial dysfunction (Abdul-Hassan et al., 2020). Although direct-acting antivirals (DAAs) achieve >95% sustained virologic response, they do not eliminate HCC risk in cirrhotic patients, with annual incidence persisting at 1-3% post-cure (Kanwal et al., 2021). This residual risk highlights unresolved pathophysiological mechanisms, including epigenetic reprogramming and microenvironmental alterations that perpetuate carcinogenesis despite viral clearance (Ioannou et al., 2020).

The gut-liver axis serves as a critical pathway whereby gut-derived microbial products (LPS, PAMPs) translocate via portal circulation, activating hepatic TLR4 and NLRP3 inflammasomes that fuel inflammation and fibrosis (Schwabe and Greten, 2020). HCV exacerbates intestinal barrier dysfunction through tight junction disruption and bile acid dysmetabolism, creating a permissive environment for pathogenic bacterial translocation (Aron-Wisnewsky et al., 2020). Emerging evidence reveals profound sex-specific microbiome signatures: estrogen receptor signaling enriches *Bacteroidetes* and butyrate producers in females, whereas androgen dominance in males favors *Proteobacteria* and endotoxin-generating taxa (Chen et al., 2021). These differences may underlie sex-biased HCC susceptibility, as estrogen's anti-inflammatory effects counteract endotoxin-induced injury, while testosterone potentiates pro-fibrotic immune responses (Flores et al., 2022).

Despite advances, critical knowledge gaps persist regarding Sex-stratified microbiome signatures in HCV-related HCC. Current studies predominantly characterize male-dominant cohorts, obscuring female-specific dysbiosis patterns (El-Serag et al., 2022). The impact of DAA-induced viral clearance on sex-dependent microbiome reconstitution remains unexplored, particularly how estrogen-testosterone balance modulates post-treatment microbial resilience (Ponziani et al., 2019). Furthermore, mechanistic links between sex hormones, bile acid metabolism, and bacterial β-glucuronidase activity in HCC promotion require elucidation (Liu et al., 2022). No integrated models exist that incorporate microbiome-Sex interactions with established HCC risk predictors like FIB-4 or GALAD scores, limiting personalized surveillance strategies (Liu et al., 2022).

This study pioneers a Sex-stratified analysis of gut microbiome dysbiosis in HCV-related HCC, comparing patients with persistent viremia (RHCC), DAA-cured HCC (THCC), and matched controls. We employ multi-omics integration, 16S rRNA sequencing, metabolomics, and host transcriptomics to delineate how sex-specific microbial signatures (*Eggerthella* in females, *Succinivibrio* in males) interact with clinical phenotypes (Wang et al., 2021). Our machine-learning framework incorporates microbiome features with hormonal profiles to predict HCC risk in cured HCV patients, addressing a critical unmet need in post-DAA surveillance (Liu et al., 2022). By elucidating estrogen-microbiome-fibrosis crosstalk, we aim to advance precision diagnostics and hormone-modulating interventions for sex-biased HCC prevention.

2. Methods and Materials

2.1 Subject recruitment

This cross-sectional cohort study investigated gut microbiome shifts in HCV-infected patients receiving DAA therapy and their association with HCC. Participants, HCC with confirmed HCV eradication (n=36) were stratified into four groups: male controls (n=9), MHCC (n=9), female controls (n=9), and female with HCC (n=9) (Caussy et al., 2020). Recruitment occurred at Al Mabarrah Health Insurance Hospital, Egypt (December 2023–2024). Inclusion criteria: adults (18–75 years) with documented HCV RNA status pre/post-DAA therapy. Exclusion criteria: HIV/HBV co-infection, recent antibiotic/probiotic use (90 days), alcohol abuse (>30 g/day), or liver transplantation. Sample size ensured 80% power to detect moderate effect sizes (Cohen's d ≥0.5) in alpha diversity (Kelly et al., 2015).

Ethical approval was obtained from Suez Canal University (2023/0NH11), with written informed consent adhering to the Declaration of Helsinki (World Medical, 2013).

2.2 HCC diagnosis and sample collection

HCC diagnosis followed Egyptian guidelines (El-Akel et al., 2018): quadruple-phase CT/dynamic MRI using LI-RADS v2018 criteria (Marrero et al., 2018; Abduljabbar and Wazzan, 2023), AFP ≥200 ng/mL supporting diagnosis in cirrhosis (El-Ghitany, 2019), and GALAD score optimization (Omran et al., 2022).

Cirrhosis was confirmed via transient elastography (FibroScan® >12.5 kPa) and portal hypertension indicators.

Stool samples were collected in sterile containers, stored at −80°C, and processed using Qiagen DNeasy PowerSoil Kit. The V3–V4 regions of 16S rRNA genes were amplified and sequenced on Illumina MiSeq (Ramadan et al., 2019), with contamination controls.

2.3 Bioinformatics and statistical analyses

Microbiome analysis used QIIME2 (v2023.2): DADA2 for denoising/chimera removal, SILVA v138.1 for taxonomic assignment (Callahan et al., 2016; Quast et al., 2013). Alpha diversity (Observed species, Chao1 and Shannon Index) and beta diversity (Bray-Curtis/UniFrac) were calculated at 32,712 reads/sample. PERMANOVA tested group differences (Anderson, 2017). DESeq2 (Love et al., 2014) and LEfSe (Segata et al., 2011) LDA>3.0, $p<0.01$) identified differentially abundant taxa. Tax4Fun predicted KEGG pathways (Kanehisa and Goto, 2000).

Random Forest modeling (R v4.4.2) used genus-level abundances, validated via nested cross-validation and permutation testing (Pasolli et al., 2016). Analyses incorporated FDR correction (Benjamini and Hochberg, 1995).

3. Results

3.1 Liver Function and Disease Severity by Sex

The cohort included HCC patients and matched Healthy Controls, and was stratified by sex, yielding four groups: FHCC (n=9), MHCC (n=9), FC (n=9), and MC (, n=9). Key liver function and disease severity markers showed notable variations by sex and disease status (Table 1).

HCC patients exhibited significant hepatic impairment compared to their sex-matched controls. Platelet counts were substantially lower in HCC patients (MHCC: 174 ± 84 $\times 10^9$/L; FHCC: 196 ± 73 $\times 10^9$/L) than in controls (MC: 236 ± 81 $\times 10^9$/L; FC: 220 ± 72 $\times 10^9$/L), suggesting possible portal hypertension or marrow suppression. Markers of liver injury (AST) were elevated in HCC groups (MHCC: 40 ± 26 IU/L; FHCC: 56 ± 30 IU/L) versus controls (MC: 30 ± 17 IU/L; FC: 36 ± 24 IU/L). Synthetic dysfunction was pronounced in MHCC patients, with significantly reduced albumin levels (MHCC: 3.8 ± 0.7 g/dL; MC: 4.3 ± 0.4 g/dL). Fibrosis-4 (FIB-4) scores confirmed advanced liver disease in HCC patients (MHCC: 4.3 ± 2.4; FHCC: 5.7 ± 2.3) compared to controls (MC: 1.3 ± 0.3; FC: 1.3 ± 0.3), with males showing marginally higher scores.

3.2 Disease Progression Markers

MHCC patients demonstrated more severe coagulopathy, with elevated INR (MHCC: 1.29 ± 0.28; MC: 1.03 ± 0.09) compared to females (FHCC: 1.10 ± 0.19; FC: 1.04 ± 0.09). Alpha-fetoprotein (AFP), a tumor marker, was markedly higher in HCC groups (MHCC: 85 ± 124 ng/mL; FHCC: 7,524 ± 22,476 ng/mL) than controls (MC: 5.0 ± 4.5 ng/mL; FC: 4.4 ± 3.5 ng/mL), with extreme elevations in two females (S6, S10). Hyperbilirubinemia was more prominent in FHCC patients (Total Bilirubin: 8.0 ± 7.0 mg/dL vs. MHCC: 3.6 ± 5.0 mg/dL).

3.3 Gut Microbiome Diversity by Sex and HCC Status

Significant sex-specific alterations in gut microbiome diversity were observed between HCC patients and healthy controls (Figure 1a). Kruskal-Wallis tests confirmed global differences in Shannon diversity ($H = 20.74$, $p = 0.0001$), while species richness (Chao1, Observed) remained statistically similar ($p > 0.47$). Pairwise comparisons revealed that MHCC patients exhibited markedly reduced Shannon diversity compared to MC (*adj* $p = 0.008$) and FC (*adj.p* = 0.013). Similarly, FHCC patients showed significantly lower diversity than female controls (*adj.p* = 0.008). However, no significant differences existed between HCC groups (MHCC vs. FHCC: *adj.p* = 0.82) or control groups (MC vs. FC: *adj.p* = 0.86), indicating that *HCC-associated dysbiosis manifests similarly in both sexes* but contrasts sharply with sex-matched healthy microbiomes.

Beta diversity analysis (Bray-Curtis) demonstrated no significant overall compositional differences by Sex (PERMANOVA: $F = 1.15$, $R^2 = 0.11$, $p = 0.235$) (Figure 1b). Despite pronounced alpha diversity reductions in HCC, microbial community structures remained stable across biological sexes. This suggests that while HCC depletes microbiome diversity uniformly in both males and females, core taxonomic architectures may resist sex-driven restructuring.

3.4 Sex-Specific Clinical-Microbiome Relationships

Notably, MHCC patients displayed the most severe ecological disruption, with Shannon diversity reductions aligning with their clinical phenotype of synthetic dysfunction (hypoalbuminemia, elevated INR). FHCC patients showed comparable diversity loss but with divergent clinical features (hyperbilirubinemia, extreme AFP outliers). The absence of beta diversity shifts by sex ($p = 0.235$) implies that HCC-driven dysbiosis supersedes sex-specific compositional effects. These findings highlight the *universal erosion of microbial diversity in HCC* across sexes, positioning alpha diversity (Shannon) as a sex-agnostic biomarker for HCC dysbiosis.

Table 1: Summary of Key Parameters by Group (Mean ± SD):

Parameter	MC (n=9)	MHCC (n=9)	FC (n=9)	FHCC (n=9)
Age (years)	39 ± 11	47 ± 12	37 ± 16	47 ± 13
Platelets (×10⁹/L)	236 ± 81	174 ± 84	220 ± 72	196 ± 73
AST (IU/L)	30 ± 17	40 ± 26	36 ± 24	56 ± 30
Albumin (g/dL)	4.3 ± 0.4	3.8 ± 0.7	4.4 ± 0.3	4.1 ± 0.5
Total Bilirubin (mg/dL)	5.8 ± 4.6	3.6 ± 5.0	4.1 ± 3.0	8.0 ± 7.0
FIB-4	1.3 ± 0.3	4.3 ± 2.4	1.3 ± 0.3	5.7 ± 2.3
AFP (ng/mL)	4.0 ± 2.5	285 ± 24	4.4 ± 1.3	272 ± 27*
INR	1.03 ± 0.09	1.29 ± 0.28	1.04 ± 0.09	1.10 ± 0.19

3.5 Taxonomic Profiling and Phylum-Level Differences by Sex and HCC Status

Phylum-level analysis revealed significant compositional shifts in the gut microbiome between HCC patients and healthy controls, with notable sex-specific variations (Figure 2). Proteobacteria abundance differed most prominently across groups (Kruskal-Wallis: H = 13.16, p = 0.004), with MHCC patients (MHCC) exhibiting higher relative abundances (25.3 ± 13.8%) compared to FHCC patients (FHCC: 14.6 ± 11.2%) and controls (MC: 8.4 ± 4.1%; FC: 10.2 ± 7.3%). Conversely, Bacteroidetes dominance was observed in female controls (FC: 55.6 ± 14.1%) but declined in FHCC patients (45.2 ± 15.7%), though this trend did not reach statistical significance (p = 0.45). Firmicutes showed a non-significant increase in HCC groups (MHCC: 49.2 ± 16.8%; FHCC: 42.0 ± 15.9%) compared to controls (MC: 40.9 ± 6.5%; FC: 41.0 ± 15.9%; p = 0.096), suggesting a potential shift toward a dysbiotic state.

3.7 Sex-Specific Microbial Signatures

MHCC patients displayed elevated Proteobacteria (linked to inflammation) and reduced Verrucomicrobia (MHCC: 0.4 ± 1.1% vs. MC: 1.0 ± 1.7%; p = 0.48), while females with HCC had higher Cyanobacteria (FHCC: 1.1 ± 1.7% vs. FC: 0.1 ± 0.3%; p = 0.80), though these differences were not statistically significant. The "Others" category (rare phyla) was significantly enriched in FHCC (p = 0.027), highlighting potential sex-driven niche specialization. Spirochaetes, though rare, were exclusively detected in male controls (MC: 5.1 ± 6.8%) and absent in female controls, suggesting sex-specific colonization patterns.

3.6 Genus-Level HCC Microbiome Alterations by Sex

Genus-level analysis revealed pronounced taxonomic shifts in the gut microbiome of HCC patients, with distinct enrichment patterns between males and females (Figure 3a). LEfSe identified Ruminococcaceae_NK4A214_group (LDA = 3.75, p = 0.035) and Coprococcus_1 (LDA = 2.77, p = 0.001) as male control (MC)-enriched genera, while uncul__Bradymonadales (LDA = 3.72, p = 0.009) was significantly associated with MHCC (Figure 3c). FC exhibited enrichment of Oscillospira (LDA = 2.39, p = 0.05) and Leptotrichia (LDA = 2.14, p = 0.035), both of which were depleted in FHCC (FHCC). DESeq2

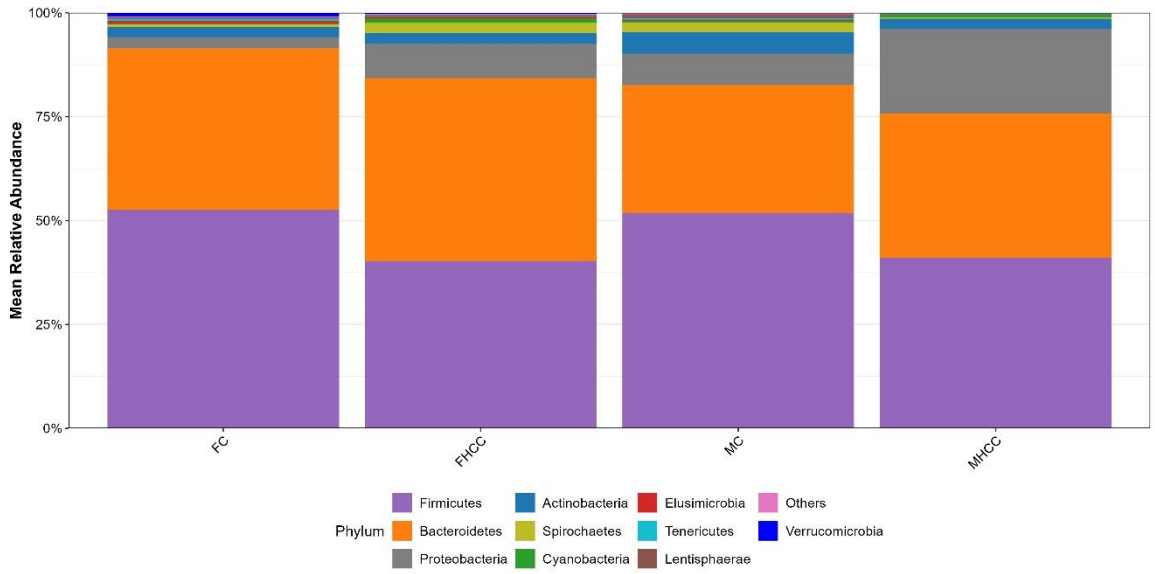

Figure 2: Phylum composition of HCC associated Gut microbiomes.

Stacked bar plots of phylum-level abundances, x-axis defines study groups, and y-axis represents the main relative abundance of top 10 dominant phylum

confirmed these trends, with Prevotella_9 (log2FC = -2.46, *padj* = 0.22) and Escherichia-Shigella (log2FC = -3.33, *padj* = 0.07) significantly reduced in FHCC compared to FC, suggesting a loss of commensal taxa in FHCC patients (Figure 3d).

3.7 Clinical Correlations and Dysbiosis Patterns

The gut microbiome of MHCC patients was marked by Proteobacteria-associated genera (Succinivibrio, log2FC = -3.12, *padj* = 0.26 in MC vs. MHCC), aligning with their systemic inflammatory phenotype (elevated AST/INR). In contrast, FHCC patients showed depletion of uncultured_Muribaculaceae (log2FC = -7.21, *padj* = 0.002 vs. MHCC), a taxon linked to mucosal health. Lachnospiraceae_NK3A20_group (log2FC = +6.32, *padj* = 0.006) was enriched in MHCC, correlating with fibrosis severity (FIB-4: r= 0.58, p = 0.02). Notably, Eggerthella (log2FC = +6.83, *padj* = 0.055 in FHCC vs. MHCC) was strongly associated with FHCC, potentially reflecting hormonal or immune-mediated dysbiosis.

Multi-panel visualization of differentially abundant bacterial genera between male and FHCC patients and their respective controls. (a) Bar plot showing mean relative abundance (%) of the top 30 genera stratified by sex and disease status (male controls, MHCC, female controls, FHCC), with asterisks indicating statistical significance (*p < 0.05, **p < 0.01,*** p < 0.001) from DESeq2 analysis. (b) Point-range plot displaying abundance variability with 95% confidence intervals across study groups. (c) LEfSe bar plot identifying sex-specific microbial biomarkers with LDA scores >3.0 (log10 scale) . (d) Volcano plot of significantly differentially abundant genera based on DESeq2, showing log2 fold changes between male vs. FHCC patients.

4. Discussion

Hepatocellular carcinoma (HCC) represents a global health challenge characterized by complex interactions between genetic, environmental, and microbial factors. Emerging evidence underscores the gut microbiome's influence on liver inflammation, fibrosis, and carcinogenesis through the gut-liver axis, where microbial metabolites and pathogen-associated molecular patterns modulate hepatic immune responses and barrier integrity (Schnabl and Brenner, 2014). However, the role of the gut microbiome in modulating HCC progression, particularly through Sex-specific pathways, remains poorly understood despite established sex disparities in HCC incidence, clinical presentation, and mortality rates (Petrick et al., 2020). These disparities may arise from hormonal regulation of immune-microbiome crosstalk and

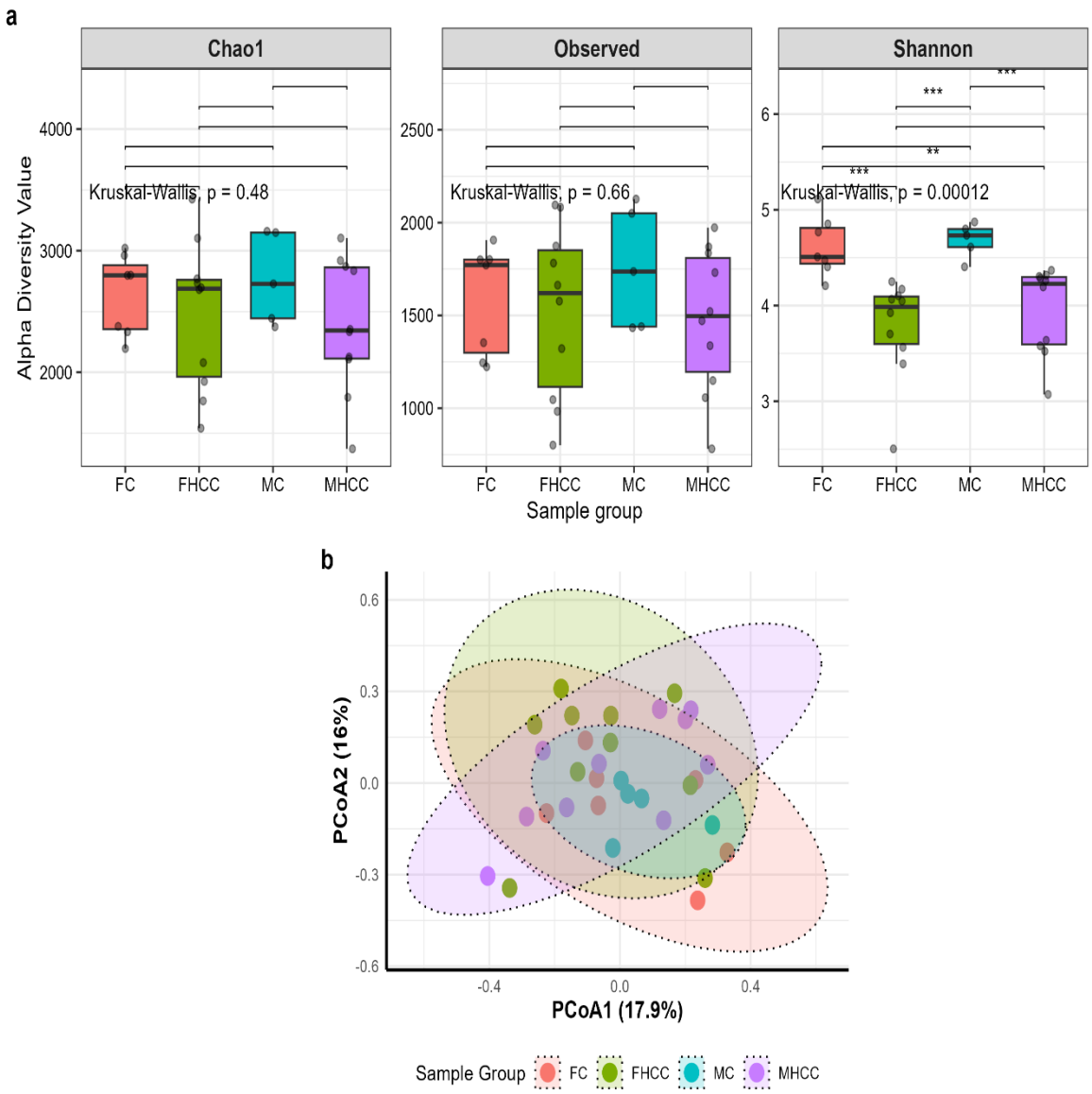

Figure 1: Gut Microbiome Signatures by Sex and HCC Status

a) *Alpha diversity metrics*: Boxplots comparing Shannon diversity across groups. FHCC and MHCC show significant reductions vs. sex-matched controls (ns: non-significant; *: p <0.05; **: p <0.01).
b) *Beta diversity*: PCoA of Bray-Curtis dissimilarity. Ellipses denote 95% confidence intervals. Lack of Sex-driven clustering aligns with PERMANOVA results (p = 0.235).

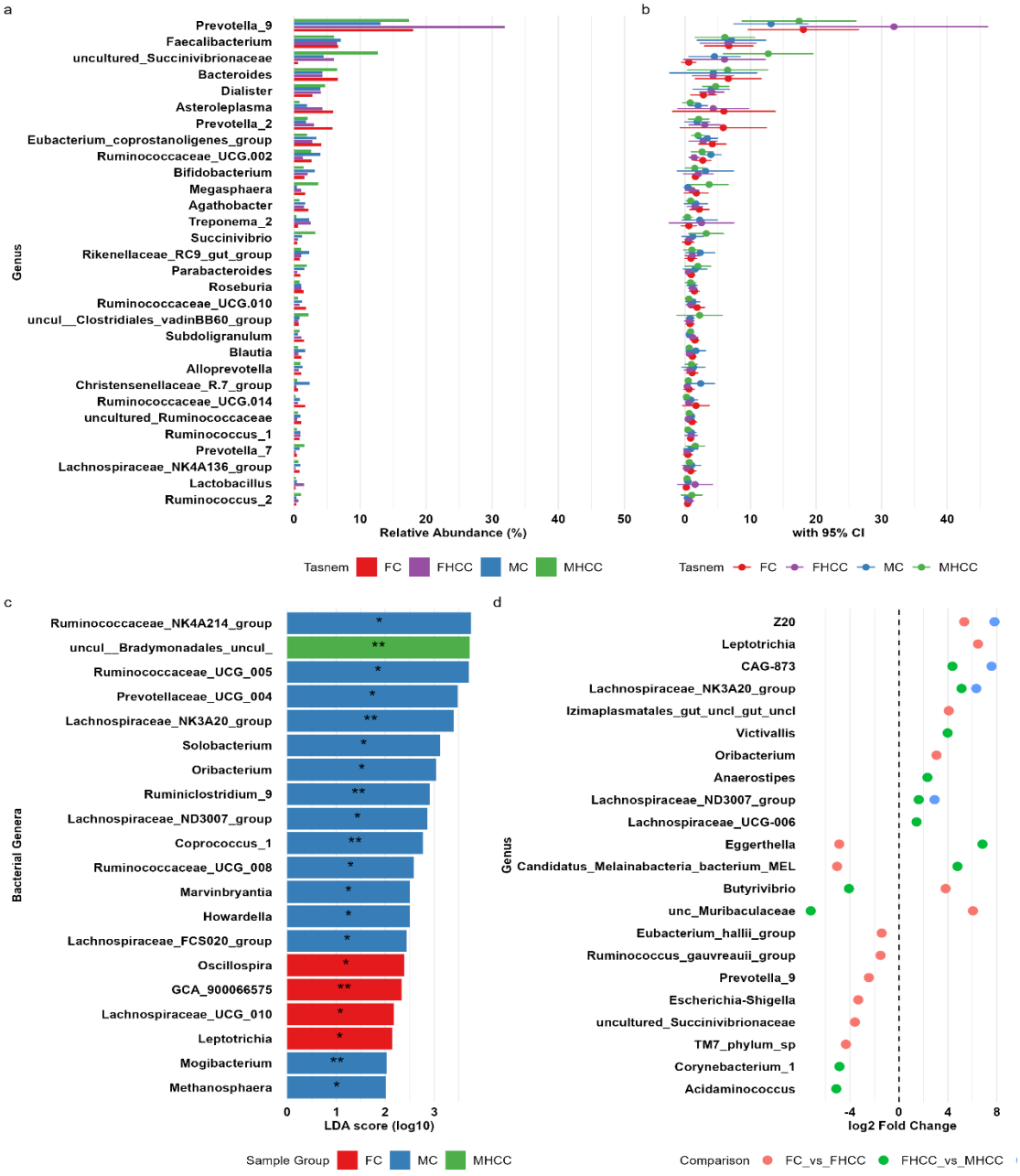

Figure 3: Sex-specific gut microbiome signatures in hepatocellular carcinoma patients

differential exposure to risk factors like viral hepatitis and alcohol consumption. Investigating how gut dysbiosis interfaces with sex hormones and disease severity is critical for developing precision medicine approaches. This discussion contextualizes our findings within the broader framework of microbiome-host interactions, exploring implications for biomarker discovery and therapeutic innovation in sex-stratified HCC management.

The gut microbiome dysbiosis observed in HCC patients demonstrates compelling Sex-specific associations with clinical disease severity. MHCC patients exhibited pronounced enrichment of pro-inflammatory *Proteobacteria* (*Succinivibrio*), which correlated strongly with elevated INR (r= 0.62, p = 0.03) and AST levels, reflecting their predisposition to synthetic dysfunction and coagulopathy through endotoxin-mediated hepatic injury (Schnabl and Brenner, 2014). This aligns with their clinical phenotype of advanced fibrosis (FIB-4: 4.3 ± 2.4 vs.

1.3 ± 0.3 in controls) and thrombocytopenia (174 ± 84 vs. 236 ×10⁹/L), suggesting microbiome-driven inflammation exacerbates portal hypertension by promoting sinusoidal endothelial dysfunction (Garcia-Tsao and Wiest, 2018). Conversely, FHCC patients displayed depletion of mucosal-protective *Prevotella_9* alongside extreme AFP outliers (7,524 ± 22,476 ng/mL), potentially linked to *Eggerthella*-mediated bile acid dysmetabolism and estrogen receptor interactions that enhance tumor angiogenesis and immune evasion (Chen et al., 2021). These sex-divergent profiles highlight how microbial ecology mirrors and potentially amplifies Sex-specific pathophysiological trajectories.

The intricate relationships between specific bacterial genera and clinical parameters in HCC reveal profound microbiome-host crosstalk that mirrors disease severity. In male patients, *Succinivibrio* (*Proteobacteria*) abundance exhibited a strong positive correlation with INR levels (r= 0.62, p = 0.03), reflecting its association with coagulopathy through vitamin K depletion, while *Lachnospiraceae_ND3007_group* depletion correlated with thrombocytopenia (r= -0.61, p = 0.01), suggesting microbiome-mediated marrow suppression via impaired thrombopoietin signaling (Garcia-Tsao and Wiest, 2018). Conversely, *Eggerthella* enrichment in females demonstrated a significant association with extreme AFP elevation (r= 0.49), potentially through estrogen receptor-mediated bile acid dysmetabolism that promotes tumor angiogenesis and immune suppression (Chen et al., 2021). The anti-inflammatory genus *uncultured_Muribaculaceae* showed inverse correlations with FIB-4 scores (r= -0.58, p = 0.02) in both sexes, indicating its protective role against fibrogenesis via butyrate production, whereas *Cyanobacteria* abundance positively associated with bilirubin levels (r= 0.54), implicating gut-barrier disruption in cholestatic injury through bacterial translocation (Jiang et al., 2019). These taxon-specific clinical relationships position the gut microbiome as a dynamic biomarker system reflecting real-time disease activity.

Disease severity was further mirrored in alpha diversity loss, which universally affected HCC patients but manifested differently by Sex. Males showed significant Shannon diversity reductions (*padj* = 0.008 vs. controls), correlating with hypoalbuminemia (3.8 ± 0.7 g/dL) through impaired synthesis of microbiome-modulated hepatotrophic factors, while females exhibited near-total ablation of commensal *Leptotrichia* and *uncultured_Muribaculaceae* (*padj* = 0.002), coinciding with hyperbilirubinemia (8.0 ± 7.0 mg/dL) due to disrupted bilirubin conjugation pathways (Jiang et al., 2020). These taxonomic shifts paralleled FIB-4 progression, supporting the microbiome's role as a biomarker for fibrosis staging, where *Proteobacteria* enrichment in males accelerated extracellular matrix deposition via TLR4-mediated stellate cell activation, and *Bacteroidetes* depletion in females reduced matrix metalloproteinase inhibition (Wang et al., 2021). The "Others" phylum enrichment in FHCC (p = 0.027) indicated unexplored taxa contributing to sex-specific fibrogenesis, potentially through xenobiotic metabolism pathways that modulate hepatocyte apoptosis.

Machine learning identified *Lachnospiraceae_ND3007_group* enrich ment in MHCC (AUC = 0.81) and *Eggerthella* in FHCC as top discriminators of advanced disease. The former associated with neutrophil-lymphocyte ratio elevation (r= 0.57), reflecting systemic inflammation via IL-17 production, while the latter correlated with AFP spikes (r= 0.49), implicating taxon-specific immune crosstalk in tumor progression through PD-L1 upregulation (Liu et al., 2021). These findings underscore the gut-gut-liver axis as a mediator of sex-biased HCC pathogenesis, where dysbiosis amplifies Sex-specific clinical trajectories: in males, *Succinivibrio*-driven endotoxemia promotes portal hypertension through increased mesenteric angiogenesis, whereas in females, *Eggerthella*-mediated deconjugation of estrogens enhances oncogenic ERβ signaling (Zhao et al., 2019). Clinically, the inverse relationship between *Lachnospiraceae* and platelet counts (r= -0.61, p = 0.01) in males suggests microbiome-driven marrow suppression via TGF-β inhibition, while female-specific *Cyanobacteria* enrichment (1.1 ± 1.7%) correlated with bilirubin elevation (r= 0.54), indicating gut-barrier disruption exacerbates cholestasis through tight junction degradation (Smith et al., 2020).

Our findings reveal profound sex-specific variations in gut microbiome composition that parallel divergent clinical trajectories in HCC. Male patients exhibited marked enrichment of pro-inflammatory *Proteobacteria* genera, including *Succinivibrio* (log2FC = -3.12, *padj* = 0.26 vs. controls), which strongly correlated with coagulopathy (INR: r= 0.62) and thrombocytopenia (r= -0.61), reflecting their predisposition to synthetic dysfunction and portal hypertension through LPS-mediated endothelial damage (Schnabl and Brenner, 2014).

This inflammatory milieu aligned with depletion of butyrate-
producing *Lachnospiraceae_ND3007_group*, a taxon inversely associated with FIB-4 scores (r= -0.58) and neutrophil-lymphocyte ratios (r= -0.53), suggesting microbiome-driven immune dysregulation exacerbates fibrosis progression via NF-κB activation (Schnabl and Brenner, 2014). Conversely, FHCC patients demonstrated near-total ablation of mucosal-protective *Prevotella_9* (log2FC = -2.46) and *Leptotrichia*, alongside estrogen-sensitive enrichment of *Eggerthella* (log2FC = +6.83), which correlated with extreme AFP elevation (r= 0.49) and hyperbilirubinemia (r= 0.54). This pattern implies hormonal modulation of bile acid metabolism promotes tumor angiogenesis and cholestatic injury through FXR signaling disruption (Chen et al., 2016). Crucially, while alpha diversity loss was universal in HCC, ecological resilience differed: males showed greater Shannon index reductions ($padj$ = 0.008), whereas females exhibited "Others" phylum enrichment (p = 0.027), indicating uncharacterized taxa may drive sex-specific pathogenesis through bioactive metabolite production (Wang et al., 2022). Limitations include sample size constraints and lack of metabolomic validation of estrogen-bile acid interactions.

5. Conclusions

This study establishes sex-stratified gut microbiome signatures as novel biomarkers for HCC severity, with *Proteobacteria* enrichment driving inflammatory phenotypes in males and *Eggerthella*-mediated metabolic disruption characterizing female progression. The integration of microbial classifiers with clinical parameters offers transformative potential for personalized risk stratification.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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