

Table S1 Comparison of clinical characteristics between the younger age group and the middle age group before surgery

Characteristic	Y.AG (n=20)	M.AG (n=13)	P value
Age (year)	42.25±4.05	53.00±6.00	<0.0001
Sex			>0.999
Female	3 (15.0%)	2 (15.4%)	
Male	17 (85.0%)	11 (84.6%)	
BMI (kg/m ²)	22.70±2.75	22.19±3.26	0.650
WBC (10 ⁹ /L)	6.19±2.05	6.12±2.58	0.842
HGB (g/L)	135.65±19.53	128.46±20.78	0.413
PLT (10 ⁹ /L)	221.55±81.53	183.77±71.46	0.181
TBIL (μmol/L)	15.22±6.36	16.96±7.09	0.524
ALB (g/L)	37.22±3.78	36.09±4.63	0.573
TRF (g/L)	2.52±0.77	2.40±0.40	0.928
PA (mg/L)	188.88±50.10	201.24±69.54	0.573
ALT (U/L)	40.50±24.65	39.38±15.61	0.524
AST (U/L)	48.90±27.82	36.46±10.49	0.353
γ-GGT (U/L)	73.5±47.04	79.31±46.44	0.624
BUN (mg/dL)	5.08±1.49	5.16±2.03	0.624
Cr (μmol/L)	72.90±13.94	77.77±12.36	0.181
HBV-DNA (/mL)	499,420.15±913,807.00	4,875,079.69±16,490,518.27	0.785
HBsAg (ng/mL)	674.77±299.15	652.12±309.87	0.842
PT (s)	12.83±1.45	12.85±0.93	0.928
INR	1.05±0.12	1.04±0.07	>0.999
AFP (ng/mL)	12,838.96±25,008.58	14,078.51±25,990.07	0.676
TCH (mmol/L)	4.45±1.08	5.24±1.58	0.147
HDL (mmol/L)	1.19±0.28	1.30±0.33	0.434
LDL (mmol/L)	3.19±1.01	3.81±1.71	0.392
Th/Ts (%)	1.91±0.70	1.98±0.82	0.957
IgG (g/L)	13.79±3.63	15.20±4.01	0.250
IgM (g/L)	1.26±0.45	1.40±0.37	0.235
IgA (g/L)	2.64±1.15	2.75±0.75	0.392
C3 (g/L)	0.85±0.17	0.82±0.22	0.334
C4 (g/L)	0.23±0.14	0.21±0.16	0.456
CK19 (%)	12.10±15.43	8.46±22.58	0.110
P53 (%)	51.00±23.37	11.92±17.86	<0.0001
Ki67 (%)	42.00±23.81	23.08±17.50	0.083
Ascites			>0.999
No	18 (90.0%)	11 (84.6%)	
Yes	2 (10.0%)	2 (15.4%)	
Smoking			>0.999
No	15 (66.7%)	10 (66.7%)	

Table S1 (continued)

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Characteristic	Y.AG (n=20)	M.AG (n=13)	P value
Yes	5 (33.3%)	3 (33.3%)	
Drinking			>0.999
No	18 (90.0%)	12 (92.3%)	
Yes	2 (10.0%)	1 (8.7%)	
Portal hypertension			>0.999
No	11 (55.0%)	10 (76.9%)	
Yes	9 (45.0%)	3 (23.1%)	
Tumor size (cm)	7.60±3.67	6.58±4.86	0.353
ICG (%)	5.36±3.06	5.35±3.39	0.928
BCLC stage			>0.999
A	12 (60.0%)	10 (76.9%)	
B	8 (40.0%)	3 (23.1%)	

P value was based on Fisher's exact test and Mann-Whitney *U*-test. Y.AG, <45 years, n=20; O.AG, >65 years, n=20. Y.AG, younger age group; O.AG, older age group; AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; BMI, body mass index; BUN, blood urea nitrogen; C3, complement factors C3; C4, complement factors C4; CK19, cytokeratin-19-fragment; Cr, serum creatinine; γ -GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; HGB, hemoglobin; IgA, immunoglobulin A; ICG, constitutional indocyanine green; IgG, immunoglobulin G; IgM, immunoglobulin M; INR, international normalized ratio; Ki67, Ki-67; LDL, low-density lipoprotein; PA, prealbumin; PLT, platelet; PT, prothrombin time; P53, Tumor suppressor p53; TBIL, total bilirubin; TCH, total cholesterol; Th/Ts, helper T cell/suppressor T cell; TRF, transferrin; WBC, white blood cell count.

Table S2 Comparison of clinical characteristics between the middle age group and the older age group before surgery

Characteristic	M.AG (n=13)	O.AG (n=20)	P value
Age (year)	53.00±6.00	67.00±3.03	<0.0001
Sex			>0.999
Female	2 (15.4%)	2 (10.0%)	
Male	11 (84.6%)	18 (90.0%)	
BMI (kg/m ²)	22.19±3.26	23.59±2.34	0.074
WBC (10 ⁹ /L)	6.12±2.58	5.78±1.46	0.785
HGB (g/L)	128.46±20.78	143.80±14.81	0.030
PLT (10 ⁹ /L)	183.77±71.46	200.80±77.81	0.650
TBIL (μmol/L)	16.96±7.09	13.79±4.52	0.250
ALB (g/L)	36.09±4.63	37.30±3.66	0.478
TRF (g/L)	2.40±0.40	2.47±0.48	0.478
PA (mg/L)	201.24±69.54	210.12±56.75	0.703
ALT (U/L)	39.38±15.61	42.70±26.24	0.758
AST (U/L)	36.46±10.49	43.10±25.25	0.434
γ -GGT (U/L)	79.31±46.44	75.65±49.32	0.676
BUN (mg/dL)	5.16±2.03	4.82±0.80	0.928
Cr (μmol/L)	77.77±12.36	76.80±12.41	0.221

Table S2 (continued)

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Characteristic	M.AG (n=13)	O.AG (n=20)	P value
HBV-DNA (/mL)	4875079.69±16490518.27	4289988.24±12362851.16	0.842
HBsAg (ng/mL)	652.12±309.87	536.79±305.57	0.235
PT(s)	12.85±0.93	12.50±1.67	0.281
INR	1.04±0.07	1.02±0.14	0.334
AFP (ng/mL)	14078.51±25990.07	391.78±1106.39	0.001
TCH (mmol/L)	5.24±1.58	4.78±1.07	0.501
HDL (mmol/L)	1.30±0.33	1.11±0.27	0.128
LDL (mmol/L)	3.81±1.71	3.50±0.97	0.870
Th/Ts (%)	1.98±0.82	1.50±0.53	0.074
IgG (g/L)	15.20±4.01	15.82±2.87	0.573
IgM (g/L)	1.40±0.37	1.42±0.45	0.842
IgA (g/L)	2.75±0.75	2.80±1.31	0.676
C3 (g/L)	0.82±0.22	0.98±1.31	0.008
C4 (g/L)	0.21±0.16	0.23±0.07	0.052
CK19 (%)	8.46±22.58	0.35±1.18	0.478
P53 (%)	11.92±17.86	22.60±34.21	0.870
Ki67 (%)	23.08±17.50	27.25±14.09	0.985
Ascites			>0.999
No	11 (84.6%)	19 (95.0%)	
Yes	2 (15.4%)	1 (5.0%)	
Smoking			>0.999
No	10 (66.7%)	16 (66.7%)	
Yes	3 (33.3%)	4 (33.3%)	
Drinking			>0.999
No	12 (92.3%)	16 (80.0%)	
Yes	1 (8.7%)	4 (20.0%)	
Portal hypertension			>0.999
No	10 (76.9%)	15 (75.0%)	
Yes	3 (23.1%)	5 (25.0%)	
Tumor size (cm)	6.58±4.86	6.17±3.81	0.870
ICG (%)	5.35±3.39	5.97±3.29	0.624
BCLC stage			>0.999
A	10 (76.9%)	11 (55.0%)	
B	3 (23.1%)	9 (45.0%)	

P value was based on Fisher's exact test and Mann-Whitney U-test. Y.AG, <45 years, n=20; O.AG, >65 years, n=20. Y.AG, younger age group; O.AG, older age group; AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; BMI, body mass index; BUN, blood urea nitrogen; C3, complement factors C3; C4, complement factors C4; CK19, cytokeratin-19-fragment; Cr, serum creatinine; γ -GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; HGB, hemoglobin; IgA, immunoglobulin A; ICG, constitutional indocyanine green; IgG, immunoglobulin G; IgM, immunoglobulin M; INR, international normalized ratio; Ki67, Ki-67; LDL, low-density lipoprotein; PA, prealbumin; PLT, platelet; PT, prothrombin time; P53, Tumor suppressor p53; TBIL, total bilirubin; TCH, total cholesterol; Th/Ts, helper T cell/suppressor T cell; TRF, transferrin; WBC, white blood cell count.

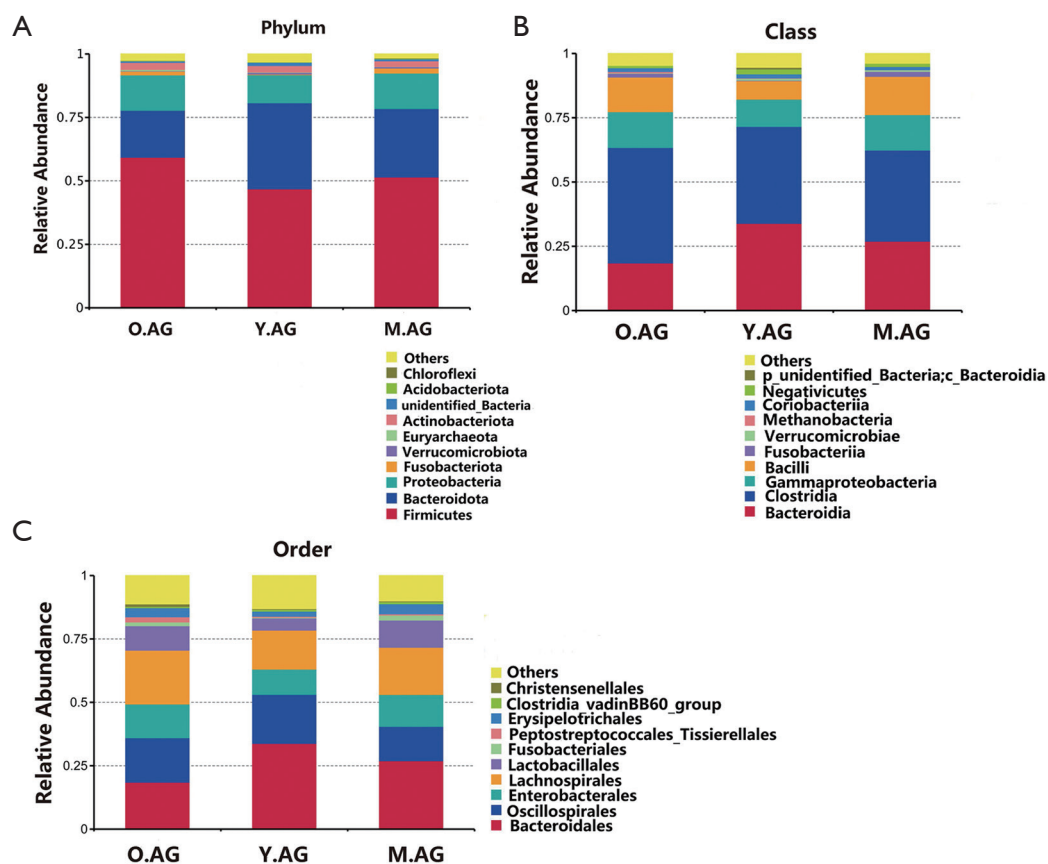


Figure S1 Relative abundance of the top 10 gut microbiota among the younger age group, the middle age group, and the older age group at the (A) phylum, (B) class, and (C) order level.

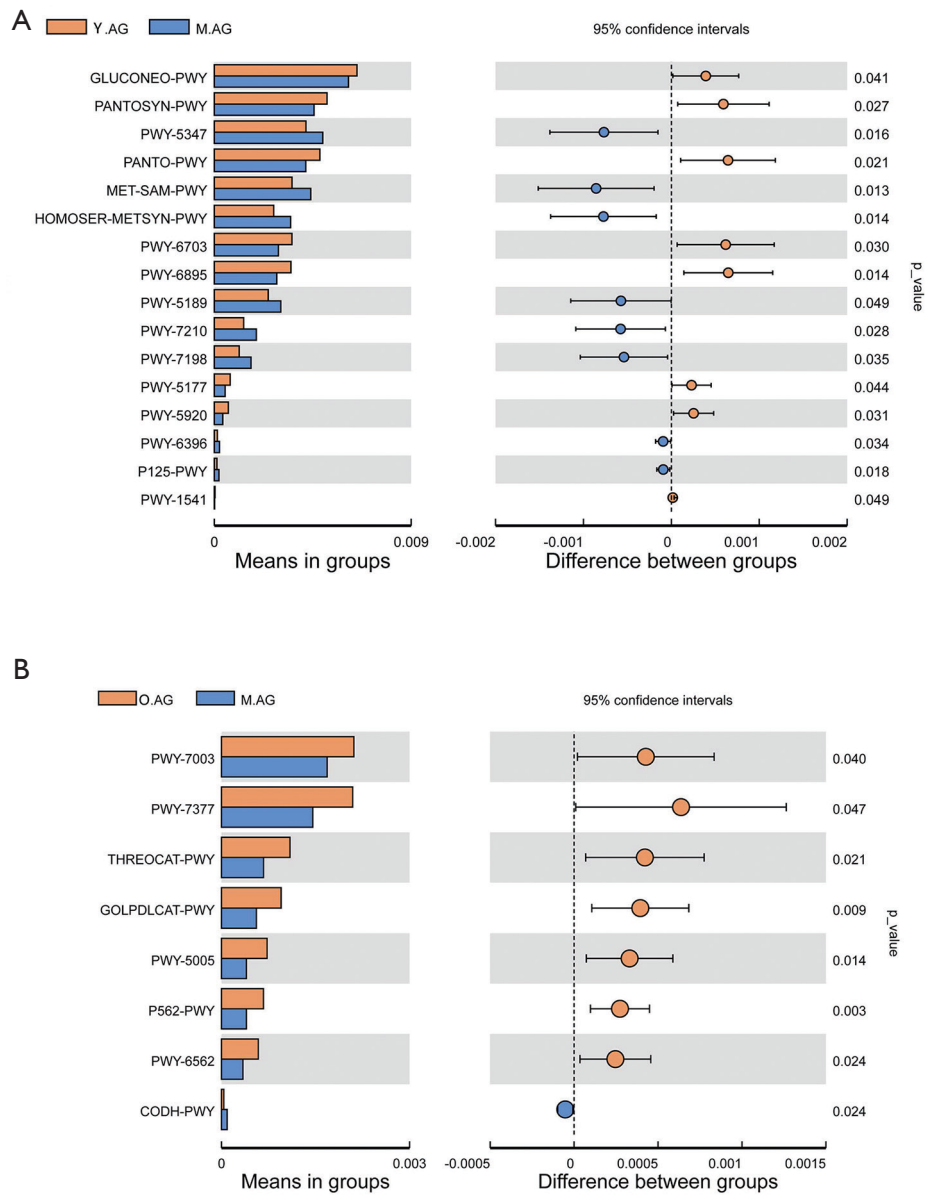


Figure S2 Functional alteration caused by gut microbiota change through PICRUST2 prediction in (A) the younger age group versus the middle age group and (B) the older age group versus the middle age group.

Table S3 Functional alteration caused by microbial change through PICRUSt2 analysis in the younger age group versus the middle age group

Pathway ID	Altered pathway	P valve
GLUCONEO-PWY	Gluconeogenesis I	0.041
PANTOSYN-PWY	Pantothenate and coenzyme A biosynthesis I	0.027
PWY-5347	Superpathway of L-methionine biosynthesis (transsulfuration)	0.016
PANTO-PWY	Phosphopantothenate biosynthesis I	0.021
MET-SAM-PWY	Superpathway of S-adenosyl-L-methionine biosynthesis	0.013
HOMOSER-METSYN-PWY	L-methionine biosynthesis I	0.014
PWY-6703	PreQ0 biosynthesis	0.030
PWY-6895	Superpathway of thiamin diphosphate biosynthesis II	0.014
PWY-5189	Tetrapyrrole biosynthesis II (from glycine)	0.049
PWY-7210	Pyrimidine deoxyribonucleotides biosynthesis from CTP	0.028
PWY-7198	Pyrimidine deoxyribonucleotides de novo biosynthesis IV	0.035
PWY-5177	Glutaryl-CoA degradation	0.044
PWY-5920	Superpathway of heme biosynthesis from glycine	0.031
PWY-6396	Superpathway of 2,3-butanediol biosynthesis	0.034
P125-PWY	Superpathway of (R,R)-butanediol biosynthesis	0.018
PWY-1541	Superpathway of taurine degradation	0.049

Table S4 Functional alteration caused by microbial change through PICRUSt2 analysis in the older age group versus the middle age group

Pathway ID	Altered pathway	P valve
PWY-7003	Glycerol degradation to butanol	0.040
PWY-7377	Cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)	0.047
THREOCAT-PWY	Superpathway of L-threonine metabolism	0.021
GOLPDLAT-PWY	Superpathway of glycerol degradation to 1,3-propanediol	0.009
PWY-5005	Biotin biosynthesis II	0.015
P562-PWY	Myo-inositol degradation I	0.003
PWY-6562	Norspermidine biosynthesis	0.024
CODH-PWY	Reductive acetyl coenzyme A pathway	0.024