

Supplementary appendix

Table A: Summary of 16s rRNA Methodologies Across Studies

Study	Sample Type	Collection	Storage	Wetlab Procedures
TPLS (2023)	Human (girls and boys)	Fecal samples collected at home, excluded if antibiotics/probiotics use in prior 3 months. Parental consent obtained.	Preserved in DNA stabilizing buffer and stored at -80°C	DNA extracted using Qiagen DNA Mini Kit, 16s rRNA V3-V4 (341F/805R); sequenced using MiSeq 2000 (illumina V3 kit)
Huang et al. (2023)	Human (girls)	Fecal samples collected	Feces immediately frozen at -80°C	DNA extracted using CTAB/SDS; 16S rRNA V4 amplified; TruSeq PCR-Free kit; sequenced on Illumina MiSeq
Huang et al. (2022)	Human (girls)	Fecal samples collected from children aged 6–10; no antibiotics or GI symptoms in prior 2 weeks	Samples stored at -80°C within 30 minutes after collection	DNA extracted using PowerSoil Kit; 16S rRNA V3–V4 amplified and sequenced (MiSeq); Greengenes database for annotation
Dong et al. (2020)	Human (girls)	Fecal samples collected at home and delivered within 12h to the hospital; participants avoided antibiotics and probiotics 2 months prior	Stored at -80°C using commercial collection kits (Micosolution, China)	DNA extracted with DNeasy PowerSoil kit; 16S rRNA V3–V4 amplified using 338F/806R primers; sequenced on Illumina MiSeq
Li et al. (2021)	Human (girls)	Fecal samples collected and stored using sterile kits; no antibiotics, probiotics prior to collection	Samples stored at -80°C	DNA extracted (method not specified); 16S rRNA sequencing (region/platform not stated)
Nguyen et al. (2023)	Animal (rats)	Stool samples collected around vaginal opening (PND 30-40)	Immediately transferred to cold storage and frozen at -80°C	DNA extracted with QIAamp DNA Stool Mini Kit; full length 16s (V1-V9) amplified with barcoded primers; sequenced using PacBio
Bo et al. (2022)	Animal (mice)	Cecal content and serum collected postmortem	Cecal contents stored for DNA extraction	16S rRNA V3–V4 sequencing (Illumina NovaSeq)
Yuan et al. (2023)	Animal (mice)	Fecal samples collected pre-puberty	Feces stored at -80°C	16S rRNA V3–V4 (MiSeq); SCFA measured via GC-MS
Wang et al. (2022)	Animal (rats)	Fecal samples collected postmortem; vaginal opening monitored; tissues collected for molecular assays	Feces frozen at -80°C	16S rRNA V3–V4 sequencing (NovaSeq 6000); GC-MS for SCFAs

Table B: Search Strategy (all fields included)

No.	Query	Results
Pubmed		
1	“Gut microbiome” or “Gut microbiota” or “Gastrointestinal microbiota” or “Gastrointestinal microbiome”	68,462
2	“Precocious puberty” or “Central precocious puberty” or “Idiopathic Central Precocious puberty” or “Early puberty”	7,679
3	# 1 and # 2	13
Web of Science		
1	“Gut microbiome” or “Gut microbiota” or “Gastrointestinal microbiota” or “Gastrointestinal microbiome”	78, 951
2	“Precocious puberty” or “Central precocious puberty” or “Idiopathic Central Precocious puberty” or “Early puberty”	6,110
3	# 1 and # 2	13
EMBASE		
1	“Gut microbiome” or “Gut microbiota” or “Gastrointestinal microbiota” or “Gastrointestinal microbiome”	117,267
2	“Precocious puberty” or “Central precocious puberty" or "Idiopathic Central Precocious puberty" or "Early puberty"	11,936
3	# 1 and # 2	17

Table C: Quality Assessment

1. Newcastle-Ottawa quality assessment scale for human studies

Quality Assessment				
Study	Selection	Comparability	Outcome	Overall Score
TPLS, 2023	★★★★★	★★	★★★	9
Huang, et al. 2023	★★★★★	★★	★★★	9
Huang, et al. 2022	★★★★★	★★	★★★	9
Li, et al. 2021	★★★★	★★	★★★	8
Dong, et al. 2020	★★★★★	★★	★★★	9

2. SYRCLE's risk of bias tool for animal studies

#	Type of bias	Domain	Judgement				
			Nguyen, 2023	Yuan, 2023	Wang, 2022	Bo, 2022	Wang, 2020
1	Selection bias	Sequence generation	Yes	Unclear	Yes	Yes	Yes
2	Selection bias	Baseline characteristics	Yes	Yes	Yes	Yes	Yes
3	Selection bias	Allocation concealment	Yes	Yes	Yes	Yes	Yes
4	Performance bias	Random housing	No	No	No	No	No
5	Performance bias	Blinding	No	No	No	No	No
6	Detection bias	Random outcome assessment	Yes	Yes	Yes	Yes	Yes
7	Detection bias	Blinding	Yes	Yes	Yes	Yes	Yes
8	Attrition bias	Incomplete outcome bias	Yes	Yes	Yes	Yes	Yes
9	Reporting bias	Selective outcome reporting	Yes	Yes	Yes	Yes	Yes
10	Other	Other sources of bias	Yes	Yes	Yes	Yes	Yes

Table D: Analysis of publication bias by Egger's test

Egger's Test							
Category		Subgroup	Bias Estimate	SE	t	df	p-value
Operational Taxonomic Units	OTU	Human	8.2579	2.4871	3.32	1	0.1862
	OTU	Animal	0.7128	0.5928	1.20	1	0.4417
Alpha Diversity	Shannon index	Human	19.3389	2.0565	9.40	2	0.0111
		Animal	-1.0969	7.3821	-0.15	2	0.8955
SCFAs	Propionic acid	Animal	-3.3443	4.1113	-0.81	1	0.5356
	Butyric acid	Animal	-5.6381	7.5182	-0.75	1	0.5904
Bacteria	<i>g_Allistipes</i>	Human	6.8078	18.9888	0.36	1	0.7808
	<i>g_Dialister</i>	Human	-3.0034	8.6306	-0.35	1	0.7868
	<i>g_Holdemania</i>	Human	-9.3276	7.3772	-1.26	2	0.3335
	<i>g_Roseburia</i>	Human	-11.0190	12.1972	-0.90	1	0.5323
	<i>g_Ruminococcus</i>	Human	-6.3723	6.9229	-0.92	1	0.5263
	<i>g_Bilophila</i>	Human	3.0794	6.4641	0.48	1	0.7170
	<i>g_Bacteroides</i>	Human	1.9705	3.9016	0.51	2	0.6637
	<i>g_Megamonas</i>	Human	1.9144	3.1551	0.61	1	0.6528

Table E: Meta-regression of potentially confounding factors

Meta-regression						
Category		Subgroup	Confounding Factor	Estimate	SE	p-value
Operational taxonomic units	OTU	Human	Year	-0.4821	0.1633	0.0032
			Taiwan vs. China	-0.0121	0.0086	0.1600
			Sample Size	-0.3145	1.4218	0.8249
		Animal	Year	0.0822	0.1994	0.6803
			Sample Size	-0.0066	0.0147	0.6513
			Rat vs. Mice	0.0583	0.6829	0.9319
SCFA	Butyric acid	Animal	Year	-0.3714	0.2546	0.1446
			Sample Size	0.0312	0.0222	0.1601
			Rats vs. Mice	-0.2801	1.1822	0.8127
	Propionic acid	Animal	Year	0.2401	0.2656	0.3660
			Sample Size	-0.0207	0.0232	0.3733
			Rats vs. Mice	0.2560	0.7892	0.7457
Alpha diversity	Shannon's index	Humans	Year	-0.1804	0.4771	0.7053
			Taiwan vs. China	-1.3202	0.8488	0.1198
			Sample Size	-0.0917	0.0715	0.1997
		Animal	Year	-0.2653	0.6527	0.6844
			Taiwan vs. China	0.7252	0.7179	0.3125
			Sample Size	-0.1378	0.1239	0.2661
			Rat vs. Mice	0.7288	0.5924	0.2186
			Danazol vs. Daidzeine	1.4264	0.8963	0.1115
			High-fat diet vs. Daidzeine	0.9723	0.7440	0.1913
	<i>g_Alistipes</i>	Human	Year	-1.7578	0.6876	0.0106

			Taiwan vs. China	-3.0174	0.3810	<.0001
			Sample Size	0.0083	0.0327	0.7991
	<i>g_Dialister</i>	Human	Year	-0.5948	0.3163	0.0601
			Taiwan vs. China	-1.1001	0.3004	0.0003
			Sample Size	0.0041	0.0110	0.7100
	<i>g_Holdemania</i>	Human	Year	-0.0948	0.3744	0.8000
			Taiwan vs. China	-1.0542	0.6467	0.1031
			Sample Size	0.0108	0.0061	0.0794
	<i>g_Roseburia</i>	Human	Year	-0.1742	0.5170	0.7362
			Taiwan vs. China	-1.2637	0.7193	0.0790
			Sample Size	0.0121	0.0089	0.1739
	<i>g_Ruminococcus</i>	Human	Year	-0.1551	0.3331	0.6415
			Taiwan vs. China	-0.9339	0.3747	0.0127
			Sample Size	0.0074	0.0066	0.2646
	<i>g_Bilophila</i>	Human	Year	-0.5080	0.1494	0.0007
			Taiwan vs. China	-0.9943	0.8215	0.2262
			Sample Size	-0.0036	0.0132	0.7832
	<i>g_Bacteroides</i>	Human	Year	0.1306	0.2050	0.5239
			Taiwan vs. China	0.7059	0.2901	0.0150
			Sample Size	-0.0027	0.0053	0.6117
	<i>g_Megamonas</i>	Human	Year	0.2303	0.1934	0.2336
			Taiwan vs. China	0.4475	0.2962	0.1308
			Sample Size	-0.0024	0.0036	0.5089

Table F: Results of multiple comparisons correction

Analysis Type	Comparison type	P-value	Bonferroni_p	FDR_p
<i>Holdemania</i>	Overall	0.0043	0.1591	0.0259
<i>Holdemania</i>	Human	0.0056	0.2072	0.0259
<i>Roseburia</i>	Overall	0.0719	1	0.0985
<i>Roseburia</i>	Human	0.0263	0.9731	0.0561
<i>Roseburia</i>	Animal	0.932	1	0.9320
<i>Alistipes</i>	Overall	0.2296	1	0.2740
<i>Alistipes</i>	Human	0.0371	1	0.0686
<i>Alistipes</i>	Animal	0.0837	1	0.1106
<i>Dialister</i>	Overall	0.0138	0.5106	0.0365
<i>Dialister</i>	Human	0.0496	1	0.0801
<i>Enterococcus</i>	Overall	0.0001	0.0037	0.0037
<i>Enterococcus</i>	Animal	0.0906	1	0.1156
<i>Ruminococcus</i>	Overall	0.365	1	0.4220
<i>Ruminococcus</i>	Human	0.0054	0.1998	0.0259
<i>Bilophila</i>	Overall	0.0008	0.0296	0.0099
<i>Bilophila</i>	Human	0.0031	0.1147	0.0229
<i>Lachnoclostridium</i>	Overall	0.0155	0.5735	0.0382
<i>Lachnoclostridium</i>	Animal	0.0707	1	0.0985
<i>Bacteroides</i>	Overall	0.0288	1	0.0561
<i>Bacteroides</i>	Human	0.0096	0.3552	0.0299
<i>Bacteroides</i>	Animal	0.82	1	0.8669
<i>Anaerostipes</i>	Overall	0.0545	1	0.0840
<i>Anaerostipes</i>	Human	0.0487	1	0.0801
<i>Anaerostipes</i>	Animal	0.8509	1	0.8745
<i>Megamonas</i>	Human	0.0073	0.2701	0.0270
<i>Gemella</i>	Overall	0.0279	1	0.0561
<i>Gemella</i>	Human	0.0276	1	0.0561
Shannon index	Overall	0.5541	1	0.6030
Shannon index	Human	0.0097	0.3589	0.0299
Shannon index	Animal	0.007	0.259	0.0270
OTU	Overall	0.057	1	0.0844
OTU	Human	0.1104	1	0.1362
OTU	Animal	0.4767	1	0.5345
Propionic acid	Overall	0.0003	0.0111	0.0056
Propionic acid	Animal	0.0111	0.4107	0.0316
Butyric acid	Overall	0.0498	1	0.0801
Butyric acid	Animal	0.0024	0.0888	0.0222