Gut microbiota in patients with Alzheimer's disease spectrum: a systematic review and meta-analysis

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ABSTRACT

Context: Gut dysbiosis has been proposed as one of pathologies in patients with Alzheimer's disease (AD) spectrum. Despite such enthusiasm, the relevant results remain substantially controversial.

Objective: A systematic review and meta-analysis were performed to investigate the differences of gut microbiota (GM) between patients with AD spectrum (including mild cognitive impairment [MCI] and AD) and healthy controls (HC).

Data sources: PubMed, MEDLINE, Scopus, and Cochrane Library from January 2000 to August 2021.

Eligibility criteria for study selection: Observational trials and pre-intervention data of intervention trials that investigated the abundance of GM in patients with AD spectrum and HC.

Data extraction and synthesis: Two reviewers independently identified articles, extracted data, and evaluated the risk of bias. The effect sizes were performed by a random-effect, inverse-variance weighted model. The effects of different countries and of clinical stages on GM abundance were also examined.

Results: 11 studies consisting of 378 HC and 427 patients with AD spectrum were included in the meta-analysis. Patients with AD, but not MCI, showed significantly reduced GM diversity as compared to HC. We also found more abundance of *Proteobacteria*, *Bifidobacterium* and *Phascolarctobacterium*, but less abundance of *Firmicutes*, *Clostridiaceae*, *Lachnospiraceae* and *Rikenellaceae* in patients with AD spectrum as compared with HC. The profiles of abundance of *Alistipes* and *Bacteroides* in HC and AD spectrum were differentially affected by countries. Finally, when considering clinical stage as a moderator, the comparisons of abundance in *Clostridiaceae* and *Phascolarctobacterium* showed large effect sizes, with gradient changes from MCI to AD stage.

Limitations: The inclusion of studies originating only from China and the U.S. was a possible limitation.

Conclusions: Patients with AD spectrum demonstrated altered GM abundance, which was differentially mediated by countries and clinical stages.

INTRODUCTION

Previous studies have suggested that amyloid-beta $(A\beta)$ peptide deposition in the brain is an early neural change in patients with Alzheimer's disease (AD) [1, 2]. However, the etiopathogenesis of AD are not well explained. Recent evidence has focused on a potential role of gut microbiota (GM) in the development or exacerbation of AD [3–5].

There are thousands of microbes residing in the human gut, which involves crucial functions for individual physiology and development [6]. Moreover. accumulating evidence has revealed that the gut and central nervous system (CNS) interact with one another through the following neuro-chemical pathways. First, GM may produce and release neurotransmitters and neurotoxins such as short-chain fatty acids (SCFAs), 5HT, acetylcholine, tryptophan, and D-lactate and ammonia [7–9]. All these molecules are transmitted by the systemic circulation and then cross the blood-brain barrier (BBB) to modulate neural activities. Second, connections of enteric nervous system (ENS) and CNS is through the vagus nerve and the autonomic nervous system [10]. Upon activation of ENS, it receives signals from GM, and then affects the gut cells and regulates anti-inflammatory effects of the peripheral immune system [11, 12]. Finally, GM is involved in the modulation of immune system through the synthesis and release of pro-inflammatory cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor-alpha [13, 14]. Interestingly, previous studies have found that GM affects the host's maturation of the neuroendocrine, nervous, and immune system; hence, the gut-brain axis plays an important role in the bidirectional communications between the ENS and CNS [15-17]. Notably, compelling evidence has proposed that any disturbance in these routes would potentially be associated with the AD occurrence [18, 19].

More recently, the changes in diversity and equilibrium of GM have attracted much attention in many neurological and psychiatric disorders. When the intestinal ecosystem is abnormally altered, the composition of GM becomes imbalanced (i.e., dysbiosis). This dysbiotic pattern prompts the host to establish a disease-related microbial community, leading to leaky intestine and BBB, as well as bacterial translocation [20]. Animal studies have demonstrated that gut dysbiosis is involved in the pathogenesis of AD [21, 22]. Studies from clinical settings have also explored the composition of GM in the patients with AD spectrum, including mild cognitive impairment (MCI) and AD [23–28]. Several GM strains were reported to be associated with the cognitive functions and neuropsychiatric symptoms in patients with AD [29]. Furthermore, it has been suggested that probiotics supplementation may be an effective dietary intervention for individuals with AD [30, 31] and other conditions, such as polycystic ovarian syndrome [32] and major depressive disorder [33].

It is interesting to note that the composition of GM is distinct from country to country. For instance, a previous study from U.S. showed an alteration of GM in patients with AD, comprising increased Bacteroidetes and reduced Actinobacteria in the phylum level [26]. In contrast, Zhuang and colleagues demonstrated opposite results in Chinese patients with AD (i.e., reduced Bacteroidetes and increased Actinobacteria) [27]. In addition, the magnitudes of gut dysbiosis have been reported to be different between patients with MCI and AD. Most of the existing literature revealed that patients with AD, but not MCI, demonstrated significantly reduced GM diversity compared to healthy older adults [25, 26, 29]. However, there was a study reporting similar GM diversity and abundance in patients with MCI and AD [24]. Thus, it remains unclear whether different clinical stages lead to different magnitudes of gut dysbiosis.

To the best of our knowledge, no statistical review of GM structure in patients with AD spectrum has been performed. Therefore, the purpose of this study was two-fold. First, we aimed to determine the differences of GM diversity and abundance between the patients with AD spectrum and healthy controls (HC). Second, we further examined the potential effects of different countries and clinical stages on GM abundance.

METHODS

Literature search

This meta-analysis followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [34]. We conducted a comprehensive literature in search PubMed. MEDLINE, Scopus, and Cochrane Library electronic databases from January 2000 to August 2021, with combinations of the following terms: ("Alzheimer's disease" OR "dementia" OR "mild cognitive impairment" OR "cognitive dysfunction") AND ("microbiota" OR "gut microbiota" OR "microbiome"). Moreover, the reference lists of the selected articles or reviews were also included as additional studies.

Eligibility criteria

Two authors (CCH and CHC) independently screened and identified the full texts that met the following inclusion criteria: (1) they were peer-reviewed articles written in English; (2) GM diversity and abundance was compared between patients with AD spectrum and HC; (3) GM was derived from stool samples; (4) only preintervention data were collected from the intervention studies; (5) the GM strains were investigated by at least three studies; (6) adequate statistical data (e.g., mean, standard deviation, p values, median, maximum, minimum, etc.) to estimate effect sizes. Studies of case reports, systematic reviews and animal research were excluded.

Outcome measures

The primary outcomes consisted of GM diversity (including α diversity and β diversity) and differences of GM abundance between the patients with AD spectrum and HC. The secondary outcomes consisted of the effects of different countries and clinical stages on GM abundance.

Data extraction

The necessary data of each study regarding the number of participants, age, body mass index, diabetes mellitus, dietary assessments, diversity and abundance of GM, etc. were extracted by CCH and checked by CHC. Median, minimum, maximum, or 95% confidence interval (CI) from 5 studies were estimated from the bar graphs [25–28, 35]. Discrepancies with study criteria or data coding were resolved by debate and consensus.

Risk of bias assessment

Two authors (CCH and CHC) independently assessed the risk of bias in each included study using the Risk of Bias Assessment Tool for Nonrandomized Studies (RoBANS) [36], which evaluates six possible sources of bias: selection of participants, confounding variables, measurement of exposure, blinding of outcome assessments, incomplete outcome data, and selective outcome reporting. Disagreements were resolved by consensus or by consultation with a third author.

Effect size calculations

The Comprehensive Meta-Analysis Version 3 software (Biostat Inc., Englewood, NJ, USA) was applied to calculate the effect sizes with a random-effect, inversevariance weighted model. Postulating that departures from Gaussian distributions were not serious, we used previously reported conversion equations [37] to estimate means and standard deviation from median, maximum and minimum. Hedges' g effect sizes were derived from the mean differences between groups of AD spectrum and HC, divided by the pooled standard deviation of these groups. Heterogeneity across each study was evaluated using Q-statistic and I². Additionally, the inclusion of outliers may result in bias and significantly influence the pooled effect sizes [38, 39]. We defined the outliers with the following criteria: 1) for which the upper boundary of the 95% CI is lower than the lower boundary of the overall effect CI (i.e., extremely small effect sizes); (2) for which the lower boundary of the 95% CI is higher than the upper boundary of the overall effect CI (i.e., extremely large effect sizes).

Potential publication bias of each GM abundance was quantitatively assessed by Begg and Mazumdar rank correlation [40] and Egger's regression intercept tests [41]. Moreover, the Duval and Tweedie's trim and fill method was used to correct for non-normal distribution of effect sizes potentially due to the file drawer problem. The significant levels were set at p < 0.05.

Availability of data

Data available on request from the corresponding authors.

RESULTS

Study selection and characteristics

By the comprehensive literature search, 164 relevant articles were yielded when duplications were excluded. After the review of the titles and abstracts, 14 studies were potentially eligible for our meta-analysis. After carefully examining the full texts, three additional studies were removed: two did not provide sufficient data [42, 43] and one did not report common GM strains as other studies [44]. Therefore, the remaining 11 articles were included in the final meta-analysis (Figure 1). Table 1 summarizes the clinical and demographic characteristics of the 11 studies. These studies were performed in China [23–25, 27, 29, 35, 45, 46] and U.S. [26, 28, 47], with a total of 378 HC and 427 patients with AD spectrum (AD = 251, MCI = 124, aMCI = 52).

Primary outcomes: *α* diversity and β diversity

Among the indices of α diversity, Shannon index and Simpson index were most frequently measured in our included articles. There were no significant differences of α diversity between HC and AD spectrum (Figure 2). However, when patients with AD spectrum were divided into those with MCI and AD, the results showed that AD but not MCI demonstrated significantly reduced α diversity as indexed by Shannon index (Hedges' g = 0.237; 95% CI = 0.023 to 0.451;

Ste	ıdy			НС						AD spect	rum		Dietary	
	ntry	Ν	Sex (M/F)	Age	BMI	DM ^a	Stage	N	Sex (M/F)	Age	BMI	DM ^a	check	Genetic analysis
Vogt et al.	U.S.	25	7/18	69.3 ± 7.5	26.1 [24.3, 33.2] ^b	2 (8)	AD	25	8/17	71.3 ± 7.3	26.0 [22.9, 29.1] ^b	2(8)	NR	16S rRNA gene sequencing using Illumina DNA extraction method: NR
(2017)					[,]						[]			MiSeq platform $(2 \times 250$ -bp) Region: V4
														Pipeline analysis: Mothur Database: Greengenes
Zhuang et al.	China	43	23/20	69.7 ± 9.2	NR	5 (11.6)	AD	43	23/20	70.1 ± 8.8	NR	7 (16.3)	NR	16S rRNA gene sequencing using Illumina DNA extraction method: Power Soil Kit
(2018)														MiSeq platform (2 x 300-bp) Region: V3-V4 Pipeline analysis: QIIME
Haran	U.S.	51	8/43	80.3 ± 10.2	NR	11 (21.6)	AD	24	4/20	84.7 ± 8.1	NR	5 (20.8)	Yes	Database: RDP DNA extraction method: PowerMag soil DNA
et al. (2019)														isolation kit NextSeq 500 sequencing system (2 x 150-bp) Region: NR
														Pipeline analysis: KneadData Database: NCBI bacterial genomes k-mer
Li et al.	China	30	13/17	63.9 ± 5.1	24.0 ± 2.9	2 (6.7)	MCI	30	12/18	65.4 ± 7.6	23.2 ± 2.9	3 (10)	NR	16S rRNA gene sequencing using Illumina DNA extraction method: QIAamp DNA Stool
(2019)														Mini Kit MiSeq platform (2 x 300-bp) Region: V3-V4
							AD	30	15/15	66.3 ± 5.1	23.0 ± 3.5	2 (6.7)		Pipeline analysis: NR Database: NR
Liu et al. (2019)	China	32	16/16	76.9 ± 9.4	22.2 ± 2.3	1 (3.1)	MCI ^c	32	14/18	70.5 ± 11.0	22.4 ± 2.6	3 (9.4)	NR	16S rRNA gene sequencing using Illumina DNA extraction method: DNA extraction kit MiSeq platform
							AD	33	19/14	74.9 ± 11.4	22.0 ± 1.3	7 (21.2)		Region: V3-V4 Pipeline analysis: QIIME
Vagpal	U.S.	6	2/4	65.2 ± 3.7	NR	NR	MCI	11	3/8	64.3 ± 7.7	NR	NR	NR	Database: Greengenes 16S rRNA gene sequencing using Illumina
et al. (2019)														DNA extraction method: QiaAmp PowerFeca DNA kit MiSeq platform (2 x 300-bp)
														Region: V4 Pipeline analysis: QIIME
Hou et al.	China	47	22/25	71.7 ± 6.7	23.6 ± 3.3	3 (6.8)	AD	30	17/13	71.9 ± 6.9	23.7 ± 4.8	7 (23.3)	Yes	Database: Greengenes 16S rRNA gene sequencing using Illumina DNA extraction method: E.Z.N.A Stool
(2021)														Extraction Kit MiSeq platform (2 x 300-bp)
														Region: V3-V4 Pipeline analysis: UPARSE
Liu et al.	China	22	9/13	72.7 ± 8.05	22.1 ± 2.3	1 (4.5)	MCI ^c	20	12/8	68.8 ± 11.2	22.8 ± 2.3	2 (10)	NR	Database: Greengenes 16S rRNA gene sequencing using Illumina DNA extraction method: DNA extraction kit
(2021)														MiSeq platform Region: V3-V4
														Pipeline analysis: QIIME Database: Greengenes
Sheng et al. 2021)	China	38	15/23	66.8 ± 5.1	24.0 ± 3.3	3 (7.9)	CI ^d	14	4/10	73.2 ± 7.9	23.4 ± 3.0	3 (21.4)	NR	16S rRNA gene sequencing using Illumina DNA extraction method: QIAamp DNA Stoo Mini Kit
														MiSeq platform (2 x 300-bp) Region: V3-V4
7hone	China	50	21/20	62 5 ± 4 0	24 2 ± 2 1	ND	MCI	75	26/20	62 0 + 4 1	24.7 + 2.0	ND	Vac	Pipeline analysis: NR Database: RDP 165 rPNA gang sequencing using Illumina
Zhang et al. 2021)	China	52	24/28	62.5 ± 4.0	24.2 ± 3.1	NR	MCI	75	36/39	62.0 ± 4.1	24.7 ± 2.9	NR	Yes	16S rRNA gene sequencing using Illumina DNA extraction method: Power Fecal DNA Isolation Kit
														HiSeq platform Region: V4

														Pipeline analysis: QIIME Database: NR
Zhou et	China	32	14/18	71.1 ± 5.9	21.7 ± 1.5	4 (12.5)	AD	60	24/36	72.8 ± 7.3	22.1 ± 1.7	10	NR	16S rRNA gene sequencing using Illumina
al.												(16.7)		DNA extraction method: QIAamp DNA Stool
(2021)														Mini Kit
														MiSeq platform (2 x 250-bp)
														Region: V3-V4
														Pipeline analysis: Mothur
														Database: RDP

Abbreviations: HC: healthy control; MCI: mild cognitive impairment; AD: Alzheimer's disease; BMI: Body Mass Index; DM: diabetes mellitus; M: male; F: female; NR: not reported; QIIME: Quantitative Insights Into Microbial Ecology; RDP: Ribosomal Database Project. ^aDM was presented as n (%); ^bBMI was presented as median [interquartile range]; ^camnestic MCI; ^athe patients consisted of MCI (*n* = 8) and AD (*n* = 6).

p = 0.030; n = 5) and Simpson index (Hedges' g = 0.395; 95% CI = 0.116 to 0.674; p = 0.005; n = 3).

Among all the included articles except for three studies [27, 35, 47], seven indicators of β diversity were assessed (Table 2). The principal coordinate analyses based on both Weighted UniFrac distance and Unweighted UniFrac distances were most frequently measured. In terms of Weighted UniFrac distance, three studies revealed significant differences [24, 29, 46], while four studies revealed no significant differences between HC and AD spectrum [23, 25, 28, 45]. In terms of Unweighted UniFrac distances, two studies revealed significances [24, 29] while four studies revealed no

significant differences between HC and AD spectrum [23, 25, 28, 45]. In brief, the findings were inconsistent in our included studies.

Primary outcome: overall effect sizes by disease

In terms of the phylum level (Figure 3), the results showed more abundance of *Proteobacteria* (Hedges' g = -0.349; 95% CI = -0.604 to -0.095; p = 0.007; n = 6) in AD spectrum versus HC. No significant difference was observed for *Firmicutes* between AD spectrum and HC (p = 0.833; n = 8). After the exclusion of two outliers [24], a significantly less abundance of *Firmicutes* (Hedges' g = 0.538; 95% CI = 0.224 to





Study	β diversity	Findings	Statistic value
Vogt et al.	NMDS of Weighted UniFrac distances	A significant difference in gut microbial composition between AD and HC	<i>p</i> < 0.001
e	NMDS of Unweighted UniFrac distances	A significant difference in gut microbial composition between AD and HC	p < 0.005
(2017)	NMDS based on Bray-Curtis dissimilarity	A significant difference in gut microbial composition between AD and HC	p < 0.001
	PCoA of Weighted UniFrac distances	A significant difference in gut microbial composition among AD, MCI and HC	p = 0.001
Li et al.	PCOA of weighted UniFrac distances	No significant difference in gut microbial composition between AD and MCI	NR
(2019)	PCoA of Unweighted UniFrac distances	A significant difference in gut microbial composition among AD, MCI and HC	p = 0.001
	FCOA OF Offweighted Official distances	No significant difference in gut microbial composition between AD and MCI	NR
		No significant difference in gut microbial composition among AD, MCI ^a and HC	NR
Liu et al.	PCoA of Weighted UniFrac distances	No significant difference in gut microbial composition among AD, MCI ^a and HC	NR
	PCoA of Unweighted UniFrac distances	A significant difference in gut microbial composition between AD and HC	p = 0.017
(2019)	PCoA based on Bray-Curtis dissimilarity	A significant difference in gut microbial composition between AD and MCI ^a	p = 0.005
		A significant difference in gut microbial composition between MCI ^a and HC	p = 0.012
Nagpal et al.	PCoA of Weighted UniFrac distances	No significant difference between MCI and HC	NR
(2019)	PCoA of Unweighted UniFrac distances	No significant difference between MCI and HC	NR
Hou et al.	PCoA of Weighted UniFrac distances	No significant difference in gut microbial composition between AD and HC	p = 0.233
	PCoA of Unweighted UniFrac distances	No significant difference in gut microbial composition between AD and HC	p = 0.065
(2021)	PCoA based on Bray-Curtis dissimilarity	A slight difference in gut microbial composition between AD and HC	p = 0.039
Shang at al	PCoA of Weighted UniFrac distances	A marginal difference in gut microbial composition between CI ^b and HC	p = 0.053
Sheng et al.	PCoA of Unweighted UniFrac distances	No significant difference in gut microbial composition among CI ^b , SCD and HC	NR
(2021)	PCoA based on Bray-Curtis dissimilarity	A significant difference in gut microbial composition between CI ^b and HC	p = 0.047
Zhang et al. (2021)	PCoA of Weighted UniFrac distances	A significant difference in gut microbial composition between MCI and HC	<i>p</i> = 0.008
7hou at al	PCoA of Weighted UniFrac distances	A significant difference in gut microbial composition between AD and HC	p = 0.026
Zhou et al.	PCoA of Unweighted UniFrac distances	A significant difference in gut microbial composition between AD and HC	p < 0.001
(2021)	PLS-DA	A clear difference in gut microbial composition between AD and HC	NR

Abbreviations: HC: healthy control; MCI: mild cognitive impairment; AD: Alzheimer's disease; SCD: subjective cognitive decline; ACE: Abundance-based Coverage Estimator; NMDS: Non-metric multidimensional scaling; PCoA: Principal Coordinate Analysis; PLS-DA: Partial Least Squares Discriminant Analysis. ^aamnestic MCI; ^bthe patients consisted of MCI (n = 8) and AD (n = 6).

0.853; p = 0.001; n = 6) was observed in AD spectrum versus HC. The abundance of *Bacteroidetes* and *Actinobacteria* did not show obvious difference between AD spectrum and HC.

In terms of the class level (Figure 4), the abundance of *Bacteroidia*, *Clostridia*, and *Gammaproteobacteria* did not show significant differences between AD spectrum and HC.

In terms of the order level (Figure 5), the differences of abundance in *Bacteroidales*, *Clostridiales*, and *Enterobacteriale* were not significant in patients with AD spectrum as compared with HC.

In terms of the family level (Figure 6), the Hedges' g effect size was 1.061 with 95% CI = 0.555 to 1.568 (p < 0.001, n = 4) for the *Clostridiaceae*, suggesting a less abundant level of this GM strain in AD spectrum versus HC. The difference of the abundance in *Lachnospiraceae* was not significant between AD spectrum and HC (p = 0.763, n = 7). After the exclusion of two outliers [24], we discovered a less abundant level of *Lachnospiraceae* (Hedges' g = 0.632; 95% CI = 0.402 to 0.862; p < 0.001; n = 5) in AD spectrum versus HC. The abundance of *Rikenellaceae* did not show obvious differences between these two groups (p = 0.459; n = 4). After the exclusion of one outlier [26], the

pooled effect size was 0.797 (95% CI = 0.305 to 1.289; p = 0.002; n = 3), suggesting less abundant of this GM strain in AD spectrum versus HC. No obvious differences were found for *Bacteroidaceae*, *Enterobacteriaceae*, and *Ruminococcaceae* between these two groups.

In terms of the genus level (Figure 7), a more abundant level of *Phascolarctobacterium* (Hedges' g = -0.852; 95% CI = -1.348 to -0.357; p = 0.001; n = 5) was found in AD spectrum versus HC. The abundance of *Bifidobacterium* did not show obvious differences between AD spectrum and HC (p = 0.728; n = 4). After the exclusion of one outlier [26], a significantly more abundant level of *Bifidobacterium* (Hedges' g = -0.608; 95% CI = -0.886 to -0.330; p < 0.001; n = 3) was detected in AD spectrum versus HC. The abundance of *Alistipes, Bacteroides*, and *Blautia* did not show significant differences between these two groups.

Secondary outcome: effect sizes by country

The pooled effect size for *Bacteroides* was not significant in the comparison between HC and AD spectrum (Figure 7). However, when country was considered as a moderator, the pooled effect sizes for U.S. (n = 2) and for China (n = 6) were -0.781 (with 95% CI from -1.301 to -0.26, p = 0.003) and 0.027 (with 95% CI from -1.194 to 1.249, p = 0.965), respectively. In brief, compared with HC, the American patients with AD spectrum showed more abundance of *Bacteroides*, but such as pattern was not found for the Chinese patients (Table 3).

It was also interesting to note that in terms of *Alistipes*, the effect size for U.S. was -1.035 (with 95% CI from

-1.461 to -0.609, p < 0.001; n = 2), suggesting more abundant of this GM strain for American patients with AD spectrum. However, the overall effect size for China was 0.792 (with 95% CI from 0.287 to 1.296, p = 0.002; n = 3), suggesting less abundant of this GM strain for Chinese patients with AD spectrum (Table 3).



Figure 2. Forest plots of Shannon index (A) and Simpson index (B) in the comparisons between healthy controls (HC) and Alzheimer's disease (AD) spectrum. Patients with AD spectrum consisted of mild cognitive impairments (MCI) and AD.

		U.S.		China					
	Hedges' g	95% CI	р	Hedges' g	95% CI	р			
P_Bacteroidetes	-0.257	[-2.086, 1.572]	0.783	0.983	[-0.108, 2.075]	0.077			
P_Firmicutes	0.455	[-0.411, 1.301]	0.308	-0.237	[-1.231, 0.758]	0.641			
G_Alistipes	-1.035	[-1.461, -0.609]	< 0.001	0.792	[0.287, 1.296]	0.002			
G_Bacteroides	-0.781	[-1.301, -0.260]	0.003	0.027	[-1.194, 1.249]	0.965			
G_Phascolarctobacterium	-1.562	[-2.104, -1.021]	< 0.001	-0.519	[-0.828, -0.211]	0.001			

Table 3. Summary of effect sizes with 95% CI when country is considered as a moderator.

Abbreviations: CI: confidence interval; P: phylum; G: Genus. The effect sizes were reported only when the number of investigations \geq 2 in both countries.

Study name	Hedges's g	Lower limit	Upper limit	p-Value		Hedges's	s g and 9	5% CI	
/ogt et al. 2017_AD	0.693	0.131	1.256	0.016	1	1	1-	H 1	1
Zhuang et al. 2018_AD	-0.798	-1.233	-0.362	0.000		- L - A	- 1 T	10	I
Li et al. 2019 MCI	-0.795	-1.315	-0.276	0.003			- 1		I
Li et al. 2019_AD	-0.793	-1.312	-0.274	0.003			- 1		I
전 동안 이 것 이 것 같아요. 이 것 이 것 이 것 이 것 이 것 이 것 이 것 이 것 이 것 이									I
Pooled effect size	-0.434	-1.113	0.244	0.210		1 7			
					-4.00 More abunda	-2.00 nt in patient	0.00 s	2.00 Less abu	4.0 ndant in pa
Heterogeneity: Q = 21.558, I square = 8	86.084, p < 0.00	1							
B Phylum_Bacteroidetes									
Study name	Hedges's g	Lower limit	Upper limit	p-Value		Hedges's	g and 95	5% CI	
Vogt et al. 2017_AD	-1.149	-1.739	-0.559	0.000		-	- _	_ 1	I
Zhuang et al. 2018_AD	0.732	0.299	1.165	0.001			` -		
Li et al. 2019_MCI	2.950	2.223	3.676	0.000				-	
Li et al. 2019_AD	3.126	2.376	3.876	0.000				- I ·	
Liu et al. 2019_MCI	-0.384	-0.872	0.105	0.124					
Liu et al. 2019_AD	0.135	-0.346	0.616	0.581			-		
Nagpal et al. 2019_MCI	0.719	-0.255	1.694	0.148			Ŧ		
Liu et al. 2021_MCI	-0.512	-1.116	0.092	0.097					
Pooled effect size	0.683	-0.268	1.634	0.159					
Pooled effect size, remove outliers	0.003	-0.208	0.629	0.675					
					-4.00	-2.00	0.00	2.00	4.00
Heterogeneity: Q = 146.958, I square =	95.237, p < 0.00	01			More abunda	nt in patient	8	Less abu	ndant in pa
C Phylum_Firmicutes	95.237, p < 0.00 Hedges's g	D1	Upper limit	p-Value	More abunda	nt in patient Hedges's	200		ndant in pa
C Phylum_Firmicutes			Upper limit 1.374	p-Value 0.005	More abundai		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD	Hedges's g	Lower limit			More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI	Hedges's g 0.806	Lower limit	1.374	0.005	More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_AD	Hedges's g 0.806 -2.275	Lower limit 0.238 -2.919	1.374 -1.630	0.005 0.000	More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI	Hedges's g 0.806 -2.275 -1.507	Lower limit 0.238 -2.919 -2.074	1.374 -1.630 -0.939	0.005 0.000 0.000	More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD	Hedges's g 0.806 -2.275 -1.507 0.257	Lower limit 0.238 -2.919 -2.074 -0.229	1.374 -1.630 -0.939 0.743	0.005 0.000 0.000 0.300	More abundar		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_AD Nagpal et al. 2019_MCI	Hedges's g 0.806 -2.275 -1.507 0.257 0.503	Lower limit 0.238 -2.919 -2.074 -0.229 0.015	1.374 -1.630 -0.939 0.743 0.991	0.005 0.000 0.000 0.300 0.044	More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_AD Nagpal et al. 2019_MCI Liu et al. 2021_MCI Liu et al. 2021_MCI	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028	1.374 -1.630 -0.939 0.743 0.991 0.862	0.005 0.000 0.000 0.300 0.044 0.863	More abundar		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size	Hedges's g 0.806 -2.275 -1.507 0.503 -0.083 0.360 1.220 -0.086	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 0.573 -0.889	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717	0.005 0.000 0.000 0.300 0.044 0.863 0.239	More abunda		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083 0.360 1.220	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 0.573	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868	0.005 0.000 0.000 0.300 0.044 0.863 0.239 0.000	-4.00		200		ndant in pa
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size	Hedges's g 0.806 -2.275 -1.507 0.503 -0.083 0.360 1.220 -0.086	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 0.573 -0.889	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717	0.005 0.000 0.300 0.044 0.863 0.239 0.000 0.833	-	Hedgos's	e g and 95	5% CI	
Heterogeneity: Q = 146.958, I square = C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_AD Li u et al. 2019_AD Nagpal et al. 2019_ACI Li u et al. 2019_ACI Sheng et al. 2021_CI Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 102.819, I square =	Hedges's g 0.806 -2.275 -1.507 0.503 -0.603 0.360 1.220 -0.086 0.538	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 -0.573 -0.889 0.224	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717	0.005 0.000 0.300 0.044 0.863 0.239 0.000 0.833	-4.00	Hedgos's	e g and 95	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size, remove outliers	Hedges's g 0.806 -2.275 -1.507 0.503 -0.603 0.360 1.220 -0.086 0.538	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 -0.573 -0.889 0.224	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717	0.005 0.000 0.300 0.044 0.863 0.239 0.000 0.833	-4.00	Hedgos's	e g and 95	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size, remove outliers Heterogeneity: Q = 102.819, I square =	Hedges's g 0.806 -2.275 -1.507 0.503 -0.603 0.360 1.220 -0.086 0.538	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 -0.573 -0.889 0.224	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717	0.005 0.000 0.300 0.044 0.863 0.239 0.000 0.833	-4.00	Hedgos's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Li ut al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Sheng et al. 2021_CI Sheng et al. 2021_CI Pooled effect size Pooled effect size Pooled effect size Pooled effect size Study name	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083 0.380 1.220 -0.086 0.538 93.192, p < 0.00	Lower limit 0.238 -2.919 -0.229 0.015 -1.028 -0.239 0.573 -0.239 0.573 -0.239 0.224	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717 0.853	0.005 0.000 0.000 0.300 0.044 0.863 0.239 0.000 0.833 0.001	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogtet al. 2017_AD L et al. 2019_MCI L et al. 2019_MCI Sheng et al. 2021_CI Pooled effect size Pooled effect siz	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083 0.360 1.220 -0.086 0.538 93.192, p < 0.00 Hedges's g	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -0.239 0.573 -0.239 0.573 -0.239 0.573 -0.239 0.224 0.224	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717 0.853	0.005 0.000 0.000 0.300 0.044 0.803 0.239 0.000 0.833 0.001	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD i et al. 2019_MC1 i et al. 2019_MC1 i u et al. 2019_MC1 Ju et al. 2019_MC1 Ju et al. 2021_MC1 Sheng et al. 2021_C1 Pooled effect size Pooled effect size Pooled effect size D Phylum_Proteobacteria Study name Ju et al. 2019_MC1 Ju et al. 2019_MC1 Ju et al. 2019_AD	Hedges's g 0.806 -2.275 -1.507 0.257 0.003 0.360 1.220 0.086 0.538 93.192, p < 0.00 Hedges's g 0.000 -0.417	Lower limit 0 238 -2 519 -2 074 -0 229 0 015 -1 028 -0 239 0 .573 -0 .889 0 .224 01 Lower limit -0 .484 -0.903	1.374 -1.630 -0.393 0.743 0.991 0.862 0.959 1.868 0.717 0.853 Upper limit 0.484 0.069	0.005 0.000 0.300 0.44 0.863 0.239 0.000 0.833 0.001 p-Value 1.000 0.092	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_CI Phylum_Proteobacteria Study name Liu et al. 2019_MCI Liu et al. 2019_MCI	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 0.300 1.220 -0.086 0.538 93.192, p < 0.00 Hedges's g 0.000	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 0.573 -0.889 0.224 01 Lower limit -0.484	1.374 -1.630 -0.393 0.743 0.991 0.862 0.959 1.868 0.717 0.853	0.005 0.000 0.300 0.863 0.239 0.000 0.833 0.001 p-Value 1.000	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogtet al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_CI Pooled effect size Pooled effe	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.080 1.220 -0.086 93.192, p < 0.00 93.192, p < 0.00 Hedges's g 0.000 -0.417 -1.380 0.000	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -1.028 -0.239 0.573 -0.839 0.224 0.224 0.224 0.224 0.239 0.224 0.484 -0.903 -2.409 -0.594	1.374 -1.630 -0.393 0.743 0.691 0.659 1.868 0.717 0.853 Upper limit 0.484 0.069 -0.311 0.594	0.005 0.000 0.300 0.044 0.863 0.239 0.000 0.833 0.001 p-Value 1.000 0.092 0.011 1.000	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogt et al. 2017_AD i et al. 2019_MC1 i et al. 2019_MC1 i u et al. 2019_MC1 i u et al. 2019_MC1 Ju et al. 2021_MC1 Sheng et al. 2021_C1 Pooled effect size Pooled effect size Pooled effect size Study name Ju et al. 2019_MC1 Ju et al. 2021_MC1 Ju et al. 2021_MC1 Study name Ju et al. 2019_MC1 Ju et al. 2019_MC1 Ju et al. 2021_MC1 Ju et	Hedges's g 0.806 -2.275 1.507 0.257 0.003 0.360 1.220 0.006 0.538 93.192, p < 0.00 Hedges's g 0.000 -0.417 -1.330 0.000	Lower limit 0 238 -2 519 -2 074 -0 229 0 015 -1 028 -0 239 0 573 -0 889 0 224 0 1 1 Lower limit -0 484 -0 903 -2 409 -0 554 -0 554 -0 554	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717 0.853 Upper limit 0.484 0.069 -0.311 0.694 -0.004	0.005 0.000 0.000 0.044 0.863 0.239 0.003 0.833 0.001 p-Value 1.000 0.982 0.01 1.000	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name //ogt et al. 2017_AD .i et al. 2019_MCI .i et al. 2019_MCI .i u et al. 2021_CI .i u et al. 2019_MCI .i u et al. 2021_MCI .i u e	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083 0.360 1.220 -0.086 0.538 93.192, p < 0.0 Hedges's g 0.000 -0.417 -1.360 0.000 -0.443 -0.438	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -0.239 0.573 -0.239 0.573 -0.239 0.573 -0.239 0.573 -0.239 0.224 -0.309 -0.309 0.224 -0.309 -0.30	1.374 -1.830 -0.939 0.743 0.991 0.859 1.868 0.717 0.853 0.853 0.853 0.069 -0.311 0.599 -0.311 0.969 -0.311 0.904 -0.004 -0.004	0.005 0.000 0.000 0.300 0.044 0.863 0.239 0.000 0.833 0.001 p-Value 1.000 0.992 0.011 1.000 0.992 0.011	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name //ogt et al. 2017_AD .i et al. 2019_MCI .i et al. 2019_MCI .i u et al. 2021_CI .i u et al. 2019_MCI .i u et al. 2021_MCI .i u e	Hedges's g 0.806 -2.275 1.507 0.257 0.003 0.360 1.220 0.006 0.538 93.192, p < 0.00 Hedges's g 0.000 -0.417 -1.330 0.000	Lower limit 0 238 -2 519 -2 074 -0 229 0 015 -1 028 -0 239 0 573 -0 889 0 224 0 1 1 Lower limit -0 484 -0 903 -2 409 -0 554 -0 554 -0 554	1.374 -1.630 -0.939 0.743 0.991 0.862 0.959 1.868 0.717 0.853 Upper limit 0.484 0.069 -0.311 0.694 -0.004	0.005 0.000 0.000 0.044 0.863 0.239 0.003 0.833 0.001 p-Value 1.000 0.982 0.01 1.000	-4.00	Hedges's	g and 99	5% CI	4.00
C Phylum_Firmicutes Study name Vogtet al. 2017_AD L et al. 2019_MCI Sheng et al. 2021_CI Pooled effect size Pooled effect size Pooled effect size Pooled effect size Pooled effect al: tet al. 2019_MCI L et al. 2011_MCI	Hedges's g 0.806 -2.275 -1.507 0.257 0.503 -0.083 0.360 1.220 -0.086 0.538 93.192, p < 0.0 Hedges's g 0.000 -0.417 -1.360 0.000 -0.443 -0.438	Lower limit 0.238 -2.919 -2.074 -0.229 0.015 -0.239 0.573 -0.239 0.573 -0.239 0.573 -0.239 0.573 -0.239 0.224 -0.309 -0.309 0.224 -0.309 -0.30	1.374 -1.830 -0.939 0.743 0.991 0.859 1.868 0.717 0.853 0.853 0.853 0.069 -0.311 0.599 -0.311 0.969 -0.311 0.904 -0.004 -0.004	0.005 0.000 0.000 0.300 0.044 0.863 0.239 0.000 0.833 0.001 p-Value 1.000 0.992 0.011 1.000 0.992 0.011	-4.00	Hedges's	g and 99	5% CI	4.00

Figure 3. Forest plots of alterations of gut microbiota in the phylum level, including Actinobacteria (**A**), Bacteroidetes (**B**), Firmicutes (**C**), and Proteobacteria (**D**). Abbreviations: AD: Alzheimer's disease; MCI: mild cognitive impairments; CI: cognitive impairments.

Different countries did not significantly modulate the abundance of *Bacteroidetes*, *Firmicutes*, and *Phascolarctobacterium* in the comparisons between HC and AD spectrum.

Secondary outcome: Effect sizes by clinical stage

The abundance of *Proteobacteria* was increased in the patients with AD spectrum. However, the significance was only found in the comparison

Α	Class	Bacteroidia

between HC and AD (Hedges' g = -0.441, 95% CI = -0.775 to -0.108, p = 0.01; n = 2), but not in the comparison between HC and MCI (Hedges' g = -0.317, 95% CI = -0.739 to 0.106, p = 0.142; n = 4). Similar trend of abundance was found in the *Phascolarctobacterium*, revealing that the abundance of this GM was significantly increased in patients with MCI versus HC (Hedges' g = -0.763, 95% CI = -1.277 to -0.248, p = 0.004; n = 3) (Table 4).



B Class Clostridia

Study name	Hedges's g	Lower limit	Upper limit	p-Value		
Li et al. 2019_MCI	-1.717	-2.303	-1.130	0.000		
Li et al. 2019_AD	-1.168	-1.709	-0.627	0.000		
Liu et al. 2019_MCI	0.214	-0.271	0.700	0.387		
Liu et al. 2019_AD	0.675	0.180	1.169	0.007		
Liu et al. 2021_MCI	0.321	-0.277	0.919	0.293		
Sheng et al. 2021_CI	1.072	0.434	1.710	0.001		
Pooled effect size	-0.101	-0.944	0.742	0.814		
Pooled effect size, remove outliers	0.217	-0.510	0.943	0.559	-4.00	

More abundant in patients Less abundant in patients

Heterogeneity: Q = 70.062, I square = 92.863, p < 0.001

C Class_Gammaproteobacteria

Study name	Hedges's g	Lower limit	Upper limit	p-Value		Hedges	's g and §	95% CI	
Liu et al. 2019_MCI	0.090	-0.394	0.574	0.716	1	1	-	·	
Liu et al. 2019_AD	-0.361	-0.846	0.123	0.144					
Liu et al. 2021_MCI	0.038	-0.556	0.632	0.900			_+	-	
Zhang et al. 2021_MCI	-0.404	-0.759	-0.049	0.026					
Pooled effect size	-0.208	-0.464	0.047	0.110					
					-4.00	-2.00	0.00	2.00	4.00
					More abune	dant in patier	nts	Less abunda	int in patients

Figure 4. Forest plots of alterations of gut microbiota in the class level, including Bacteroidia (**A**), Clostridia (**B**), and Gammaproteobacteria (**C**). Abbreviations: AD: Alzheimer's disease; MCI: mild cognitive impairments; CI: cognitive impairments.

In contrast, we found a trend toward decreased abundance of *Clostridiaceae* in the patients with MCI (Hedges' g = 0.700, 95% CI = -0.013 to 1.413, p = 0.054; n = 2), which was more pronounced in the patients with AD (Hedges' g = 1.406, 95% CI = 1.001 to 1.810, p < 0.001; n = 2) (Table 4).

Risk of bias

The quality of the included studies is summarized in Supplementary Table 1. Each study was classified as

A Order_Bacteroidales

low risk in five criteria. In the criteria of confounding variables, all studies, except for one [23], suffered from a high-risk bias due to the potential confounding factors (e.g., body mass index, diabetes and diet) on GM structure.

Publication bias

The Begg and Mazumdar rank correlations as well as Egger's regression intercept tests confirmed that most of these meta-analysis results were not significantly



Heterogeneity: Q = 116.429, I square = 96.564, p < 0.001

B Order_Clostridiales

Study name	Hedges's g	Lower limit	Upper limit	p-Value		Hedges's	g and 9	5% CI	
Li et al. 2019_MCI	-1.717	-2.303	-1.130	0.000	1				1
Li et al. 2019_AD	-1.168	-1.709	-0.627	0.000					
Liu et al. 2019_MCI	0.214	-0.271	0.700	0.387				·	
Liu et al. 2019_AD	0.779	0.280	1.277	0.002			- H		
Liu et al. 2021_MCI	0.322	-0.290	0.933	0.303				-	
Sheng et al. 2021_CI	1.072	0.435	1.710	0.001			-		
Pooled effect size	-0.084	-0.946	0.778	0.849			\blacklozenge	►	
Pooled effect size, remove outliers	0.238	-0.509	0.986	0.532	-4.00	-2.00	0.00	2.00	4.00
					More abund	lant in patients		Less abunda	nt in patients

Heterogeneity: Q = 72.436, I square = 93.097, p < 0.001

C Order_Enterobacteriales

Study name	Hedges's g	Lower limit	Upper limit	p-Value		Hedges	's g and §	95% CI	
iu et al. 2019_MCI	0.069	-0.415	0.553	0.780			-	·	
iu et al. 2019_AD	-0.462	-0.949	0.025	0.063					
iu et al. 2021_MCI	0.035	-0.572	0.643	0.910			_	-	
lou et al. 2021_AD	-0.463	-0.922	-0.004	0.048			-		
ooled effect size	-0.229	-0.521	0.064	0.126					
					-4.00	-2.00	0.00	2.00	4.00
					More abund	lant in patier	nts	Less abunda	int in patients

Figure 5. Forest plots of alterations of gut microbiota in the order level, including Bacteroidales (**A**), Clostridiales (**B**), and Enterobacteriale (**C**). Abbreviations: AD: Alzheimer's disease; MCI: mild cognitive impairments; CI: cognitive impairments.

Table 4. Summary of effect sizes with 95% CI when	n clinical stage is considered as a moderator.
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		HC vs. MCI		HC vs. AD				
	Hedges' g	95% CI	р	Hedges' g	95% CI	р		
P_Bacteroidetes	0.680	[-0.879, 2.239]	0.393	0.693	[-0.712, 2.099]	0.334		
P_Firmicutes	-0.434	[-1.683, 0.814]	0.495	-0.063	[-1.438, 1.312]	0.928		
P_Proteobacteria	-0.317	[-0.739, 0.106]	0.142	-0.441	[-0.775, -0.108]	0.010		
C_Bacteroidia	0.707	[-1.228, 2.642]	0.474	1.323	[-0.087, 2.733]	0.066		
C_Clostridia	-0.390	[-1.648, 0.868]	0.544	-0.243	[-2.049, 1.562]	0.792		
O_Bacteroidales	0.644	[-1.372, 2.660]	0.531	1.642	[-1.207, 4.490]	0.259		
O_Clostridiales	-0.391	[-1.655, 0.874]	0.545	-0.192	[-2.099, 1.716]	0.844		
O_Enterobacteriales	0.056	[-0.323, 0.434]	0.773	-0.462	[-0.796, -0.128]	0.007		
F_Bacteroidaceae	-0.082	[-1.727, 1.564]	0.922	0.500	[-0.622, 1.621]	0.383		
F_Clostridiaceae	0.700	[-0.013, 1.413]	0.054	1.406	[1.001, 1.810]	< 0.001		
F_Enterobacteriaceae	0.278	[-0.951, 0.394]	0.417	-0.460	[-0.794, -0.126]	0.007		
F_Lachnospiraceae	-0.016	[-1.300, 1.268]	0.980	-0.058	[-1.156, 1.040]	0.917		
F_Rikenellaceae	0.716	[0.007, 1.426]	0.048	-0.086	[-2.231, 2.060]	0.937		
F_Ruminococcaceae	-0.296	[-0.673, 0.082]	0.125	0.300	[-0.856, 1.456]	0.611		
G_Alistipes	0.708	[-0.018, 1.435]	0.056	-0.374	[-1.741, 0.993]	0.592		
G_Bacteroides	-0.961	[-3.516, 1.594]	0.461	0.262	[-0.673, 1.197]	0.583		
G_Blautia	0.265	[-0.633, 1.162]	0.563	-0.370	[-0.952, 0.212]	0.213		
G_Phascolarctobacterium	-0.763	[-1.277, -0.248]	0.004	-0.953	[-2.166, 0.260]	0.124		

Abbreviations: CI: confidence interval; P: Phylum; C: Class; O: Order; F: Family; G: Genus. The effect sizes were reported only when the number of investigations \geq 2 in both diagnoses.

biased by publication errors. The adjusted Hedges' g was operated in 4 GM strains, including *Firmicutes*, *Gammaproteobacteria*, *Bacteroidales*, and *Enterobacteriale* (Supplementary Table 2).

DISCUSSION

This study conducted meta-analysis to compare GM abundance between the patients with AD spectrum and HC, and yielded four major insights into the nature of GM alterations in AD spectrum. First, patients with AD, but not MCI, exhibited decreased GM diversity as compared to HC. Second. Proteobacteria. Bifidobacterium and Phascolarctobacterium were more abundant in AD spectrum, whereas Firmicutes, Clostridiaceae, Lachnospiraceae and Rikenellaceae were less abundant in AD spectrum compared to HC. Third, the abundance of *Alistipes* was significantly increased in American patients but significantly decreased in Chinese patients as compared to HC. Altered abundance of Bacteroides was only found in the American patients but not in the Chinese patients. Finally, the abundance of Proteobacteria and Phascolarctobacterium was progressively increased from HC to AD stage, while the abundance of Clostridiaceae was gradually reduced from HC to AD stage.

To our knowledge, the present meta-analysis is the first to assess α diversity and β diversity in patients with AD spectrum. Generally, several studies have reported that alpha diversity is significantly decreased in patients with AD [25, 26] but not in patients with MCI [28, 35]. These findings were consistent with our results of metaanalysis, and there was also a trend toward a progressive decline from MCI to AD. Similarly, the decrease of α diversity was also found in other conditions, such as Parkinson's disease [48] and irritable bowel syndrome (IBS) [49]. In terms of β diversity, further exploration is obviously needed to examine between HC and AD spectrum due to the extremely inconsistent findings.

The *Proteobacteria* is a major phylum of gram-negative bacteria [50]. Of note, the *Proteobacteria* member *Escherichia coli*-derived neurotoxins are correlated with AD neuropathology and increase the release of pro-inflammatory cytokines [44]. It has also been shown that an increased level of *Proteobacteria* was associated with pro-inflammatory dietary pattern (e.g., high-fat diet), and the abundance of *Proteobacteria* increased along with worse memory dysfunction [51, 52]. Taken together, our current finding that patients with AD spectrum showed abnormally more abundance of *Proteobacteria* was supported by previous literature.

The phylum *Firmicutes* serves a connection with inflammatory effects, the modulation of metabolic function and the production of SCFAs [53, 54]. Several lines of evidences have demonstrated that decreased *Firmicutes* was associated with the development of obesity and type 2 diabetes [55, 56]. It was also important to note that insulin resistance might lead to cerebral glucose hypometabolism and enhanced A β accumulation in asymptomatic middle-aged people with increased risk of AD [57, 58]. Furthermore, the abundance of

Firmicutes was positively associated with the performance of executive function, suggesting that it is a kind of beneficial GM strain for the humans [45, 59].

The family Clostridiaceae performs a vital function on producing SCFAs, which can offer fuel sources for the host and protective effects on permeability of gut and

Study name	Hedges's a	Lower limit	Upper limit	p-Value	Heda	es's g and	95% CI	
					1 1		1	
Vogt et al. 2017_AD	-1.170	-1.762 0.689	-0.578 1.594	0.000	1			
Zhuang et al. 2018_AD Li et al. 2019_MCI	1.585	1.010	2.159	0.000	1 1		-	
Li et al. 2019_AD	1.715	1.129	2.302	0.000				
Liu et al. 2019_MCI	-1.024	-1.540	-0.509	0.000	1 1-			
Liu et al. 2019_AD	0.296	-0.187	0.780	0.229	1 1		-	
Liu et al. 2021_MCI	-0.803	-1.421	-0.184	0.011	·			
Pooled effect size	0.251	-0.645	1.148	0.583	1 1	-		1
					-4.00 -2.00	0.00	2.00	4.00
				M	lore abundant in pa	tients L	ess abunda	ant in patient
					52 53			
Heterogeneity: Q = 116.174, I square	= 94.835, p <	0.001						
B Family Clostridiaceae								
Study name	Hedges's g	Louvor limit	Linnes limit	n Volue	Hadaa	o'o a ond	0.5% (1	
Study name		Lower limit			Heage	s's g and	95% CI	
Vogt et al. 2017_AD	1.644	1.010	2.278	0.000	TT		-	
Liu et al. 2019_MCI	1.048	0.531	1.565	0.000	1 1			
Liu et al. 2019_AD	1.242	0.716	1.767	0.000	1 1			
Liu et al. 2021_MCI	0.320	-0.278	0.918	0.294	1 1			
Pooled effect size	1.061	0.555	1.568	0.000	I I	1 4		1
					-4.00 -2.00	0.00	2.00	4.00
				Mo	re abundant in pati	ents Le	ss abundar	nt in patients
Heterogeneity: Q = 9.606, I square =	68.769, p = 0.	022						
C Family_Enterobacteria	iceae							
Study name	Hednee's a	Lower limit	Unner limit	n-Value	Hadaa	s's g and	95% CI	
					nedge	ssy and	1000	
Liu et al. 2019_MCI	0.046	-0.438	0.530	0.852		-		
Liu et al. 2019_AD	-0.458	-0.944	0.029	0.065		-		
Nagpal et al. 2019_MCI	-1.360	-2.408	-0.311	0.011	+=	— <u> </u>		
Liu et al. 2021_MCI	0.036	-0.558	0.630	0.905		_		
Hou et al. 2021_AD	-0.463	-0.922	-0.004	0.048				1
Pooled effect size	-0.327	-0.684	0.031	0.073	1 1	-	1	1
						0.00	2.00	4.00
					-4.00 -2.00			
				Mo	-4.00 -2.00 re abundant in pati			nt in patients
		092		Mc				
D Family_Lachnospirace	eae		Upper limit		re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace	eac Hedges's g	Lower limit		p-Value	re abundant in pati		ss abundar	
Study name Zhuang et al. 2018_AD	eae Hedges's g 0.518	Lower limit	0.944	p-Value 0.017	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Liet al. 2019_MCI	eae Hedges's g 0.518 -1.310	Lower limit 0.092 -1.862	0.944 -0.759	p-Value 0.017 0.000	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Liet al. 2019_AD Li et al. 2019_AD	Hedges's g 0.518 -1.310 -1.267	Lower limit 0.092 -1.862 -1.815	0.944 -0.759 -0.718	p-Value 0.017 0.000 0.000	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_MCI	Hedges's g 0.518 -1.310 -1.267 0.711	Lower limit 0.092 -1.862 -1.815 0.212	0.944 -0.759 -0.718 1.211	p-Value 0.017 0.000 0.000 0.005	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD	Hedges's g 0.518 -1.310 -1.267	Lower limit 0.092 -1.862 -1.815	0.944 -0.759 -0.718 1.211 1.034	p-Value 0.017 0.000 0.000	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI	Hedges's g 0.518 -1.310 -1.267 0.711 0.544	Lower limit 0.092 -1.862 -1.815 0.212 0.055	0.944 -0.759 -0.718 1.211	p-Value 0.017 0.000 0.000 0.005 0.029	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Sheng et al. 2021_CI Poolde offect isize	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.105	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 -0.582	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_MCI Sheng et al. 2021_MCI Sheng et al. 2021_CI	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.592	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625	p-Value 0.017 0.000 0.005 0.029 0.076 0.002	re abundant in pati	ents Le	ss abundar	
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_MCI Sheng et al. 2021_MCI Sheng et al. 2021_CI	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.105	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 -0.582	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763 0.000	Hedge	ents Le	95% CI	nt in patients
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size Pooled effect size, remove outliers	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.992 0.105 0.632	Lower limit 0.092 -1.862 -1.815 0.212 -0.055 0.055 0.359 -0.582 0.402	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763 0.000	Hedge	ents Le	95% CI	nt in patients
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size, remove outliers	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.992 0.105 0.632	Lower limit 0.092 -1.862 -1.815 0.212 -0.055 0.055 0.359 -0.582 0.402	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763 0.000	Hedge	ents Le	95% CI	nt in patients
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size, remove outliers	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.992 0.105 0.632	Lower limit 0.092 -1.862 -1.815 0.212 -0.055 0.055 0.359 -0.582 0.402	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763 0.000	Hedge	ents Le	95% CI	nt in patients
Comparing the second seco	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.105 0.632 91.572, p < 0	Lower limit 0.092 -1.862 -1.815 0.212 -0.055 0.055 -0.056 0.359 -0.582 0.402	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793 0.862	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.763 0.000 Mo	Hedge Hedge 4.00 -2.00 re abundant in pati	ents Le	95% CI	nt in patients
Comparison of the second state of the sec	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.0992 0.105 0.632 91.572, p < 0 Hedges's g	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 -0.056 0.359 -0.582 0.402	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793 0.862	p-Value 0.017 0.000 0.005 0.029 0.763 0.000 Mc	Hedge Hedge 4.00 -2.00 re abundant in pati	ents Le	95% CI	nt in patients
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D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Sheng et al. 2021_CI Pooled effect size Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_AD	Hedges's g 0.518 -1.310 -1.267 0.549 0.549 0.549 0.992 0.105 0.632 91.572, p < 0	Lower limit 0.092 -1.862 -1.815 0.212 0.055 0.359 0.402 0.001 Lower limit -1.778 0.571 0.571 0.571	0.944 -0.759 -0.718 1.211 1.034 1.155 0.793 0.862 Upper limit -0.592 1.626 1.535	p-Value 0.017 0.000 0.005 0.076 0.002 0.763 0.000 Md p-Value 0.000 0.000	Hedge Hedge 4.00 -2.00 re abundant in pati	ents Le	95% CI	nt in patients
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D Family_Lachnospirace Study name Study name Zhuang et al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_MCI Liet al. 2011_CI Pooled effect size Provide offect size Pooled effect size Study name Vogt et al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_MCI Provelot effect size Provide diffect size	Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.549 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.109 1.005 0.382	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 -0.582 0.402 1.001 Lower limit -1.778 0.571 0.474 0.474	0.944 -0.759 -0.718 1.211 1.034 1.155 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535	p-Value 0.017 0.000 0.005 0.029 0.763 0.002 0.763 0.000 Mc p-Value 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.005	Hedge Hedge 4.00 -2.00 re abundant in pati	ents Le	95% CI	nt in patients
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D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Pooled effect size Pooled effect size Pooled effect size E Family_Rikenellaceae Study name Vogt et al. 2019_MCI Li et al	eae Hedges's g 0.518 -1.310 -1.267 0.549 0.549 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.109 1.005 0.336 0.336 0.797	Lower limit 0.092 -1.862 -1.815 0.212 0.056 0.359 0.402 0.001 Lower limit -1.778 0.571 0.474 0.0574 0.574 0.305	0.944 -0.759 -0.718 1.211 1.034 1.155 0.793 0.862 Upper limit -0.592 1.646 1.635 0.737 1.226	p-Value 0.017 0.000 0.029 0.076 0.002 0.0763 0.000 Mc p-Value 0.000 0.000 0.000 0.000 0.000 0.000 0.035 0.459 0.002	Hedge	s's g and 0,00 ents Le	95% C1 2.00 25% C1 95% C1 95% C1 95% C1	4.00 4.00
Control Contro Control Control Control Control Control Control Control Control Co	Hedges's g 0.518 -1.310 -1.267 0.514 0.544 0.545 0.532 91.572, p < (Lower limit 0.092 -1.862 -1.815 0.212 0.056 0.359 0.402 0.001 Lower limit -1.778 0.571 0.474 0.0574 0.574 0.305	0.944 -0.759 -0.718 1.211 1.034 1.155 0.793 0.862 Upper limit -0.592 1.646 1.635 0.737 1.226	p-Value 0.017 0.000 0.029 0.076 0.002 0.0763 0.000 Mc p-Value 0.000 0.000 0.000 0.000 0.000 0.000 0.035 0.459 0.002	Hedge	s's g and 0,00 ents Le	95% C1 2.00 25% C1 95% C1 95% C1 95% C1	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2021_MCI Pooled effect size Pooled effect size Pooled effect size E Family_Rikenellaceae Study name Vogt et al. 2019_MCI Li et al	Hedges's g 0.518 -1.310 -1.267 0.514 0.544 0.545 0.532 91.572, p < (Lower limit 0.092 -1.862 -1.815 0.212 0.056 0.359 0.402 0.001 Lower limit -1.778 0.571 0.474 0.0574 0.574 0.305	0.944 -0.759 -0.718 1.211 1.034 1.155 0.793 0.862 Upper limit -0.592 1.646 1.635 0.737 1.226	p-Value 0.017 0.000 0.029 0.076 0.002 0.0763 0.000 Mc p-Value 0.000 0.000 0.000 0.000 0.000 0.000 0.035 0.459 0.002	Hedge	s's g and 0,00 ents Le	95% C1 2.00 25% C1 95% C1 95% C1 95% C1	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Pooled effect size Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_MCI Li et al. 20	Hedges's g 0.518 -1.310 -1.267 0.549 0.549 0.992 0.105 0.632 91.572, p < 0	Lower limit 0.092 -1.862 -1.815 0.212 0.056 0.359 0.402 0.001 Lower limit -1.778 0.571 0.474 0.0574 0.574 0.305	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.228	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 Mc p-Value 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.002 Mc	Hedge	s's g and 0,00 ents Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Let al. 2019_MCI Let al. 2019_MCI Let al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_ACI Nocled effect size Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_AD Let al. 2019_	Hedges's g 0.518 -1.310 -1.267 0.549 0.549 0.549 0.992 0.105 0.632 91.572, p < 0	Lower limit 0.092 -1.862 -1.815 0.212 0.055 0.359 0.402 0.001 Lower limit 0.571 0.474 0.028 0.571 0.474 0.305 0.001 Lower limit	0.944 -0.759 -0.718 1.211 1.315 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.228 1.229	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 Mc p-Value 0.000 0.00	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2021_MCI Cooled effect size Prooled effect size F Family_Rikenellaceae Study name Wogt et al. 2021_MCI Decode effect size F Family_Rikenellaceae Construction	Area Control Con	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 0.402 0.001 Lower limit 0.474 0.571 0.474 0.574 0.474 0.458	0.944 -0.759 1.211 1.034 1.213 1.155 1.625 0.793 0.862 Upper limit 1.646 1.535 1.226 1.228 Upper limit	p-Value 0.017 0.000 0.029 0.076 0.002 0.763 0.000 Mc p-Value 0.000 0.00	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Liet al. 2019_MCI Liet al. 2019_MCI Liet al. 2019_AD Liet al. 2019_AD Liet al. 2019_AD Liet al. 2019_AD Liet al. 2019_ACI Liet al. 2019_ACI Liet al. 2019_ACI Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_ACI Liet al. 2010_ACI Liet al. 2019_ACI Liet al. 2019_ACI Liet al. 2019_ACI Liet al.	eae Hedges's g 0.518 -1.310 -1.267 0.711 0.549 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 0.382 0.336 0.797 92.328, p < 0 Redges's g 1.041 -0.802	Lower limit 0.092 -1.862 -1.815 0.212 0.055 0.359 -0.582 0.402 0.001 Lower limit 0.571 0.474 0.571 0.474 0.305 0.554 0.305 0.051 0.554 0.305 0.051 0.554 0.305 0.554 0.305 0.554 0.305 0.554 0.305 0.554 0.305 0.554 0.554 0.305 0.554 0.554 0.305 0.554 0.305 0.554 0.305 0.554 0.305 0.554 0.554 0.305 0.0554 0.0555 0.055	0.944 -0.759 -0.718 -0.718 -1.155 -1.625 -0.793 -0.793 -0.862 -0.992 -1.846 -1.626 -1.226 -1.226 -1.226 -1.228 -0.396	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.076 0.000 0.00	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Lie tal. 2019_MCI Lie tal. 2019_MCI Lie tal. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2012_AD Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_AD Lie tal. 2018_AD	Hedges's g 0.518 -1.310 -1.267 0.514 0.544 0.549 0.542 91.572, p < (Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 0.402 1.001 Lower limit -1.778 0.571 0.474 0.474 0.305 -0.028 -0.554 0.305 .001 Lower limit 0.458 -1.238 -0.862	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.226 1.226 1.226 1.226 1.226 1.228 1.229	р-Value 0.017 0.000 0.005 0.029 0.029 0.076 0.000 Мо 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.459 0.000 0.000 0.000	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Example al. 2021_CI Prooled effect size Prooled effect size Family_Rikenellaceae Study name Vogt et al. 2021_MCI Li et al. 2019_AD Example al. 2021_MCI Example al. 2021_MCI Example al. 2021_MCI Example al. 2021_MCI Prooled effect size Prooled effect size Family_Rikenellaceae Study name Vogt et al. 2021_MCI Example al. 20	eae Hedges's g 0.518 -1.310 -1.267 0.711 0.549 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.109 0.382 0.336 0.797 92.328, p < 0 Hedges's g Hedges's g 1.041 0.42 0.364 0.382 0.336 0.797	Lower limit 0.092 -1.862 -1.815 0.212 0.055 0.402 0.055 0.402 0.001 Lower limit 0.474 0.028 -0.554 0.305 0.028 -0.554 0.305 0.028 -0.554 0.305 0.028 -0.554 0.305 -1.238 -1.238 -0.862 -0.199	0.944 -0.759 -0.718 1.211 1.355 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.289 Upper limit 1.828 1.289	p-Value 0.017 0.000 0.005 0.029 0.076 0.002 0.06 0.000	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Li et al. 2019_MCI Li et al. 2019_MCI Li et al. 2019_MCI Liu et al. 2019_AD Liu et al. 2021_CI Pooled effect size Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_AD Li et al. 2017_AD Li et al. 2017_AD Li et al. 2019_AD Li	Hedges's g 0.518 -1.310 -1.267 0.514 0.544 0.549 0.542 91.572, p < (Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.359 0.402 1.001 Lower limit -1.778 0.571 0.474 0.474 0.305 -0.028 -0.554 0.305 .001 Lower limit 0.458 -1.238 -0.862	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.226 1.226 1.226 1.226 1.226 1.228 1.229	р-Value 0.017 0.000 0.005 0.029 0.029 0.076 0.000 Мо 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.459 0.000 0.000 0.000	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD Lie tal. 2019_MCI Lie tal. 2019_MCI Lie tal. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD Liu et al. 2012_AD Pooled effect size Pooled effect size Family_Rikenellaceae Study name Vogt et al. 2019_AD Lie tal. 2018_AD	2282 Hedges's g 0.518 -1.310 -1.267 0.544 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.005 0.336 0.797 = 92.328, p < 0 2282 Hedges's g -1.45 1.005 0.336 0.797 = 92.328, p < 0 200 200 200 200 200 200 200 2	Lower limit 0.092 -1.862 -1.815 0.055 0.055 0.402 0.001 Lower limit -1.778 0.574 0.574 0.474 0.542 0.574 0.305 1.001 Lower limit 0.474 0.028 -0.554 0.305 1.001	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.733 1.226 1.226 1.228 1.228 1.228 1.228 1.228 1.228 1.228	р-Value 0.017 0.000 0.005 0.029 0.076 0.002 Мо 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.0459 0.000 Мо	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD i et al. 2019_MCI i et al. 2019_MCI i et al. 2019_MCI i et al. 2019_AD i et al. 2021_MCI i et al. 2019_AD i e	eae Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.109 1.005 0.382 0.336 0.797 • • • • • • • • • • • • •	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.402 0.001 Lower limit -1.778 0.571 0.474 0.028 -0.554 0.305 1001 Lower limit 0.458 -1.238 -0.862 0.199 -0.775 0.152	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.228 1.228 1.228 1.228 1.289	p-Value 0.017 0.000 0.029 0.029 0.029 0.029 0.029 0.000 Mc 0.000 0	Hedge	erits Le	95% C1 2.00 25% C1 95% C1 2.00 2.00 2.00 2.00 2.00 2.00	4.00 4.00
D Family_Lachnospirace Study name Zhuang et al. 2018_AD i et al. 2019_MCI i et al. 2019_MCI i et al. 2019_MCI i et al. 2019_AD i et al. 2021_MCI i et al. 2019_AD i e	eae Hedges's g 0.518 -1.310 -1.267 0.711 0.544 0.992 0.105 0.632 91.572, p < 0 Hedges's g -1.185 1.109 1.005 0.382 0.336 0.797 • • • • • • • • • • • • •	Lower limit 0.092 -1.862 -1.815 0.212 0.055 -0.056 0.402 0.001 Lower limit -1.778 0.571 0.474 0.028 -0.554 0.305 1001 Lower limit 0.458 -1.238 -0.862 0.199 -0.775 0.152	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.228 1.228 1.228 1.228 1.289	р-Value 0.017 0.000 0.005 0.029 0.002 0.0763 0.000 Mc 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.0459 0.0002 Мс	Hedge 4.00 -2.00 re abundant in pati Hedge 4.00 -2.00 re abundant in pati	s's g and 0.00 ents Le	95% CI 2.00 95% CI 95% CI 95% CI 95% CI 95% CI 95% CI 95% CI	4.00 A.00 At in patients A.00 At in patients A.00 At in patients A.00 At in patients
D Family_Lachnospirace Study name 2nuang et al. 2018_AD jet al. 2019_MCI jet al. 2019_MCI jet al. 2019_AD jut al. 2021_MCI jut al. 2019_AD jut al. 2019_AD jut al. 2019_AD jut al. 2019_AD jut al. 2019_MCI jut al. 2019_MCI jut al. 2019_MCI jut al. 2019_AD jut al. 2019_AD jut al. 2019_AD jut al. 2019_MCI jut al. 2011_MCI jut al.	Hedges's g 0.518 -1.310 -1.267 0.549 0.549 0.992 0.105 0.632 91.572, p < 0	Lower limit 0.092 -1.862 -1.815 0.056 0.359 -0.582 0.402 0.001 Lower limit -1.778 0.571 0.474 0.574 0.574 0.574 0.305 0.051 Lower limit 0.474 0.055 0.305 0.01 Lower limit 0.452 0.305 0.01 0.574 0.305 0.574 0.305 0.054 0.305 0.054 0.305 0.574 0.305 0.057 0.554 0.305 0.057 0.554 0.305 0.057 0.554 0.305 0.057 0.554 0.305 0.057 0.055 0.055 0.055 0.057 0.057 0.055 0.152 0.075 0.152 0.043 0.055 0.152 0.043 0.055 0.152 0.043 0.055 0.152 0.043 0.055 0.152 0.043 0.055 0.043 0.055 0.152 0.043 0.055 0.152 0.043 0.055 0.043 0.043 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.045 0.043 0.045 0.04	0.944 -0.759 -0.718 1.211 1.035 1.625 0.793 0.862 Upper limit -0.592 1.646 1.535 0.737 1.226 1.228 1.228 1.228 1.228 1.289	р-Value 0.017 0.000 0.005 0.029 0.002 0.0763 0.000 Mc 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.0459 0.0002 Мс	Hedge	s's g and 0.00 ents Le	95% CI 2.00 95% CI 95% CI 95% CI 95% CI 95% CI 95% CI 95% CI	4.00 at in patients

Figure 6. Forest plots of alterations of gut microbiota in the family level, including Bacteroidaceae (A), Clostridiaceae (B), Enterobacteriaceae (C), Lachnospiraceae (D), Rikenellaceae (E), and Ruminococcaceae (F). Abbreviations: AD: Alzheimer's disease; MCI: mild cognitive impairments; CI: cognitive impairments.

BBB [60]. Moreover, it has been shown that *Clostridiaceae* was the producers of indole-3-propionic acid, which could prevent oxidative injuries of primary neurons from A β [61]. Decrease of *Clostridiaceae* may impair cognitive function and intrinsic brain activities

as evident by the results of resting-state functional magnetic resonance imaging [35]. Correspondingly, the family *Lachnospiraceae* is the producer of butyrate, which participates in anti-inflammatory reactions, and in turn maintains the gut barrier [62, 63]. A number of

Study name	Hedges's g	Lower limit	Upper limit	p-Value	Hedges's g and 95% CI
/ogt et al. 2017_AD	-1.288 1.109	-1.889 0.571	-0.687 1.646	0.000	
Li et al. 2019_MCI					
Li et al., 2019_AD	1.005	0.474	1.535	0.000	
Haran et al. 2019_AD	-0.848	-1.347	-0.349	0.001	
Zhang et al., 2021_MCI	0.365	0.011	0.719	0.044	
Pooled effect size	0.075	-0.784	0.933	0.865	
					-4.00 -2.00 0.00 2.00 4.00
				Mor	e abundant in patients Less abundant in patient
				WOR	e abundant în patients Less abundant în patient
Heterogeneity: Q = 60.936, I square =	= 93.436, p < 0	.001			
B Genus_Bacteroides					
Study name	Hedges's g	Lower limit	Upper limit	p-Value	Hedges's g and 95% Cl
Vogt et al. 2017_AD	-1.073	-1.658	-0.488	0.000	
Zhuang et al. 2018_AD	1.071	0.622	1.519	0.000	
Li et al. 2019_MCI	1.585	1.010	2.159	0.000	
Li et al. 2019_MCI	1.715	1.129	2.302	0.000	
Liu et al. 2019_MCI	-1.256 0.134	-1.786 -0.347	-0.725 0.615	0.000	
Liu et al. 2019_AD				0.586	
Haran et al. 2019_AD	-0.539	-1.027	-0.051	0.030	
Liu et al. 2021_MCI	-3.270	-4.188	-2.352	0.000	
Pooled effect size	-0.177	-1.139	0.784	0.718	
Pooled effect size, remove outliers	0.233	-0.644	1.109	0.603	-4.00 -2.00 0.00 2.00 4.00
				More	e abundant in patients Less abundant in patient
Heterogeneity: Q = 171.697, I square	= 95.923 n <	0.001			
	p				
C Genus_Bifidobacterium					
Study name	Hedges's g	Lower limit	Upper limit	p-Value	Hedges's g and 95% CI
Vogt et al. 2017_AD	1.336	0.731	1.941	0.000	
Li et al. 2019 MCI	-0.677	-1.191	-0.163	0.010	
-	-0.677	-1.191 -1.245	-0.163		
Li et al. 2019_AD	-0.729	-1.245	-0.213	0.006	
Li et al. 2019_AD Zhou et al. 2021_AD	-0.729 -0.476	-1.245 -0.907	-0.213 -0.045	0.006 0.031	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size	-0.729 -0.476 -0.151	-1.245 -0.907 -1.004	-0.213 -0.045 0.702	0.006 0.031 0.728	
Li et al. 2019_MCI Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers	-0.729 -0.476 -0.151	-1.245 -0.907	-0.213 -0.045	0.006 0.031	-4.00 -2.00 0.00 2.00 4.00
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size	-0.729 -0.476 -0.151	-1.245 -0.907 -1.004	-0.213 -0.045 0.702	0.006 0.031 0.728 0.001	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers	-0.729 -0.476 -0.151 -0.608	-1.245 -0.907 -1.004 -0.886	-0.213 -0.045 0.702	0.006 0.031 0.728 0.001	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square =	-0.729 -0.476 -0.151 -0.608	-1.245 -0.907 -1.004 -0.886	-0.213 -0.045 0.702	0.006 0.031 0.728 0.001	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers	-0.729 -0.476 -0.151 -0.608	-1.245 -0.907 -1.004 -0.886	-0.213 -0.045 0.702	0.006 0.031 0.728 0.001	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Ω = 33.376, I square = D Genus_Blautia Study name	-0.729 -0.476 -0.151 -0.608 91.012, p < 0	-1.245 -0.907 -1.004 -0.886	-0.213 -0.045 0.702 -0.330	0.006 0.031 0.728 0.001 More	
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD	-0.729 -0.476 -0.151 -0.608 •91.012, p < 0 Hedges's g -0.850	-1.245 -0.907 -1.004 -0.886 .001 Lower limit -1.420	-0.213 -0.045 0.702 -0.330 Upper limit -0.279	0.006 0.031 0.728 0.001 p-Value 0.004	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD	-0.729 -0.476 -0.151 -0.608 91.012, p < 0	-1.245 -0.907 -1.004 -0.886	-0.213 -0.045 0.702 -0.330	0.006 0.031 0.728 0.001 More	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_MCI	-0.729 -0.476 -0.151 -0.608 •91.012, p < 0 Hedges's g -0.850	-1.245 -0.907 -1.004 -0.886 .001 Lower limit -1.420	-0.213 -0.045 0.702 -0.330 Upper limit -0.279	0.006 0.031 0.728 0.001 P-Value 0.004 0.022 0.007	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_AD	-0.729 -0.476 -0.151 -0.608 91.012, p < 0 Hedges's g -0.850 -0.598	-1.245 -0.907 -1.004 -0.886 .001 Lower limit -1.420 -1.109	-0.213 -0.045 0.702 -0.330 Upper limit -0.279 -0.087	0.006 0.031 0.728 0.001 More p-Value 0.004 0.022	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_MCI	-0.729 -0.476 -0.151 -0.608 91.012, p < 0 Hedges's g -0.850 -0.598 -0.715	-1.245 -0.907 -1.004 -0.886 .001 .001 .1.420 -1.109 -1.231	-0.213 -0.045 0.702 -0.330 Upper limit -0.279 -0.087 -0.200	0.006 0.031 0.728 0.001 P-Value 0.004 0.022 0.007	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_AD Liu et al. 2019_AD Liu et al. 2019_AD	-0.729 -0.476 -0.151 91.012, p < 0 Hedges's g -0.850 -0.598 -0.715 0.818 0.490	-1.245 -0.907 -1.004 -0.888 .001 Lower limit -1.420 -1.109 -1.231 0.314 0.002	-0.213 -0.045 0.702 -0.330 Upper limit -0.279 -0.087 -0.200 1.323 0.0977	0.006 0.031 0.728 0.001 P-Value 0.004 0.022 0.007 0.001	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_AD Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI Liu et al. 2019_MCI	-0.729 -0.476 -0.151 -0.608 •91.012, p < 0 •91.012, p < 0 •0.850 -0.598 -0.715 .0.818	-1.245 -0.907 -1.004 -0.886 .001 Lower limit -1.420 -1.109 -1.231 0.314	-0.213 -0.045 0.702 -0.330 Upper limit -0.279 -0.087 -0.200 1.323	0.006 0.031 0.728 0.001 p-Value 0.004 0.022 0.007 0.001 0.004	e abundant in patients Less abundant in patient
Li et al. 2019_AD Zhou et al. 2021_AD Pooled effect size Pooled effect size, remove outliers Heterogeneity: Q = 33.376, I square = D Genus_Blautia Study name Vogt et al. 2017_AD Li et al. 2019_MCI Li et al. 2019_AD Liu et al. 2019_AD	-0.729 -0.476 -0.151 -0.608 • 91.012, p < 0 • Hedges's g -0.850 -0.598 -0.715 0.818 0.490 0.588 0.437	-1.245 -0.907 -1.004 -0.886 -0.886 -0.001 -1.420 -1.109 -1.231 0.314 0.002 -0.021 -0.667	-0.213 -0.045 0.702 -0.330 Upper limit -0.279 -0.087 -0.200 1.323 0.977 1.193	0.006 0.031 0.728 0.001 p-Value 0.004 0.002 0.007 0.001 0.049 0.059 0.046	e abundant in patients Less abundant in patient
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Figure 7. Forest plots of alterations of gut microbiota in the genus level, including Alistipes (A), Bacteroides (B), Bifidobacterium (C), Blautia (D), and Phascolarctobacterium (E). Abbreviations: AD: Alzheimer's disease; MCI: mild cognitive impairments.

studies have discovered that less abundance of *Lachnospiraceae* would result in insulin resistance, disruptions of homeostasis within the CNS and exacerbation of AD neuropathology [55, 64]. Hence, the *Lachnospiraceae* was considered as an advantageous GM strain.

The genus Bifidobacterium is involved in the production of acetate and γ -aminobutyric acid, which has neuroprotective effects on the hosts [65, 66]. It is worth mentioning that Bifidobacterium members have been associated with anti-inflammatory effects and reduced permeability of gut [67]. In addition, animal studies have shown that Bifidobacterium obviously alleviated the development of AD pathologies [3]. It has also been reported that probiotics with Bifidobacterium ameliorated cognitive impairments in patients with AD [68]. However, our meta-analysis exhibited an unexpected result that an increase of Bifidobacterium was found in subjects with AD spectrum. This finding might imply the potential gut-brain self-preventative mechanism to rebuild intestinal homeostasis [69]. Nevertheless, it merits future research with a larger sample size to have an in-depth investigation.

The composition and abundance of GM may be influenced by many factors such as age, geographical areas, dietary pattern, and chronic stress. In general, the random-effect sizes for Bacteroides and Alistipes in AD spectrum did not show obvious differences as compared with HC. These non-significant results were potentially owing to large heterogeneities among the included studies. When considering the country (i.e., China and U.S.) as a moderator, our meta-analysis demonstrated an overgrowth of Bacteroides and Alistipes in American patients; however, this pattern was not observed in Chinese patients. A previous meta-analysis study has found that Chinese patients with IBS did not show obvious changes of abundance in Bacteroides compared to HC; inversely, enriched abundance of Bacteroides was observed in patients with IBS from other areas such as U.S. and Finland [49]. It has also been reported that enhanced Bacteroidetes (genus Bacteroides) members might be a possible signature for AD spectrum since certain members of this phylum are opportunistic pathogens, especially Bacteroides fragilis (B. fragilis) [70]. Interestingly, B. fragilis can be divided into two strains: non-toxigenic B. fragilis (NTBF) and enterotoxigenic B. fragilis (ETBF). NTBF participates in suppression of colitis and strengthening gut barrier; in contrast, ETBF secretes B. fragilis toxins and is highly associated with inflammatory bowel disease [71, 72]. Up to the present, there has been no reasonable interpretation to account for an increased level of Alistipes abundance in patients with AD

spectrum. It will be a valuable issue for future studies to validate our meta-analysis finding.

We considered the clinical stage as another moderator in the present study. Compared to HC, Proteobacteria, and Phascolarctobacterium were gradually enhanced from MCI to AD stage. The pro-inflammatory Proteobacteria has been suggested as a predictor for AD pathogenesis [23, 35]. In contrast, Clostridiaceae was found to be progressively reduced from MCI to AD patients. The abundance of beneficial Clostridiaceae has been reported to be significantly decreased in patients with AD, suggesting that it is a distinctive biomarker in predicting the development of AD. In addition, the results of some GM strains in the comparisons of HC versus MCI and HC versus AD did not show gradient changes. The abundance of Enterobacteriales was significantly increased in patients with AD, but a little decreased in patients with MCI. This non-gradient pattern from HC to AD was in line with a previous study [25, 35]. The abundance of Rikenellaceae was significantly reduced in patients with MCI, but a little enhanced in AD patients, suggesting that the association between Rikenellaceae and AD pathogenesis needs further exploration.

In spite of these interesting findings, our study was not without limitations. First, the generalization of these results to other populations is questionable because the vast majority of included studies originated from just two countries. Second, many of the studies suffer from significant sources of bias. There were obvious statistical heterogeneities among the included studies, which could be attributed to differences in dietary pattern, geographical background, center settings, and inclusion criteria of AD spectrum including various regimens, medication doses, illness duration, etc. For example, only three studies [23, 46, 47] in our metaconducted the dietary analysis assessments. Nevertheless, we applied the random-model to estimate the effect sizes to reduce the influences of the heterogeneities on our results. Third, we manually extracted the necessary data from the bar graphs in several studies, which might lead to another type of bias. However, this procedure was performed by two reviewers with sufficient discussion and consensus. Hence, we reasoned that the direction of the statistical significance in the between-group comparisons would not be substantially affected since we performed this method uniformly across the studies. Fourth, the effects in many occasions were assessed by very few studies and thus the current results should be interpreted with cautions. It merits future research to include more studies to provide stronger evidence on this issue. Fifth, different methods of nucleic acid extraction and gene sequencing (Table 1) are also the potential biases on the

results. For example, the differences of GM diversity between HC and AD spectrum might be greater based on the V3-V4 region than those on the V4 region. However, the limited number of studies (three with V4 region and seven with V3-V4 region) impeded us to perform additional analysis.

In conclusion, we demonstrated that *Proteobacteria*, *Bifidobacterium* and *Phascolarctobacterium* were significantly higher abundant in patients with AD spectrum, whereas *Firmicutes*, *Clostridiaceae*, *Lachnospiraceae* and *Rikenellaceae* were significantly lower in patients with AD spectrum compared to HC. Moreover, the dysbiosis of these GM can be viewed as an environmental factor of the AD initiation and progression. In the future, a larger cohort study is needed to further examine the differences of GM in AD spectrum.

AUTHOR CONTRIBUTIONS

Conceived and designed the work: CCH, RN, CHC; Acquired the data: CCH, CHC; Analyzed the data: CCH, RN, CHC; Participated in the discussion and provided the comments: CCH, CCC, CWH, RN, CHC; Wrote the paper: CCH, CHC; All of the authors have read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this study.

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SUPPLEMENTARY MATERIALS

Supplementary Tables

Study	The selection of participants	Confounding variables	Measurement of exposure	Blinding of outcome assessments	Incomplete outcome data	Selective outcome reporting
Vogt et al. (2017)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Zhuang et al. (2018)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Haran et al. (2019)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Li et al. (2019)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Liu et al. (2019)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Nagpal et al. (2019)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Hou et al. (2021)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Liu et al. (2021)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Sheng et al. (2021)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Zhang et al. (2021)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Zhou et al. (2021)	Low risk	High risk	Low risk	Low risk	Low risk	Low risk

Supplementary Table 1. Bias of the 11 studies included in this meta-analysis based on RoBANS.

Abbreviation: RoBANS: Risk of Bias Assessment tool for Non-randomized Studies.

Supplementary Table 2. Publication bias assessments.

	Begg and Mazumdar rank correlation		Egger's regression intercept test		Duval and Tweedie's trim and fill		
	Tau	P value	Intercept	P value	Observed Hedges' g	Adjusted Hedges' g	
Phylum-Actinobacteria	0.500	0.308	15.608	0.353			
Phylum_Bacteroidetes	0.178	0.536	7.657	0.301			
Phylum_Firmicutes	0	1.000	-3.536	0.683	-0.086 [-0.889, 0.717]	-0.272 [-1.068, 0.524]	
Phylum_Proteobacteria	0	1.000	-1.324	0.526			
Class_Bacteroidia	0.266	0.452	14.817	0.131			
Class_Clostridia	0.133	0.707	-3.045	0.857			
Class_Gammaproteobacteria	0.167	0.734	3.750	0.265	-0.208 [-0.464, 0.047]	-0.292 [-0.568, -0.017]	
Order_Bacteroidales	0.500	0.220	21.963	0.081	1.038 [-0.392, 2.468]	0.530 [-0.881, 1.941]	
Order_Clostridiales	0.133	0.707	-2.684	0.883			
Order_Enterobacteriale	0.167	0.734	5.490	0.448	-0.229 [-0.521, 0.064]	-0.334 [-0.643, -0.025]	
Family_Bacteroidaceae	0	1.000	-8.140	0.633			
Family_Clostridiaceae	0.167	0.734	0.539	0.970			
Family_Enterobacteriaceae	-0.100	0.806	-2.793	0.349			
Family_Lachnospiraceae	0	1.000	-4.253	0.717			
Family_Rikenellaceae	0	1.000	-2.688	0.819			
Family-Ruminococcaceae	0.400	0.259	13.807	0.139			
Genus_Alistipes	0	1.000	-4.501	0.686			
Genus_Bacteroides	-0.178	0.536	-12.118	0.245			
Genus_Bifidobacterium	0	1.000	16.626	0.309			
Genus_Blautia	-0.285	0.367	2.562	0.817			
Genus_Phascolarctobacterium	-0.500	0.220	-3.835	0.326			

Note: the observed and adjusted effects sizes were reported only when missing studies were found and corrected by Duval and Tweedie's trim and fill. The Hedges' g was presented as overall effect size [lower limit, upper limit].