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Association between sphingomyelin levels and gut microbiota abundance in Alzheimer's disease: a two-sample Mendelian randomization study

Liping Wang[1†], Yuyan Ding[1†], Yu Tang[1†], Mengqi Yang[2], Zhihui Yang[1], Xiao Yang[1] and Jiazeng Xia[1,2,3*]

Abstract

Background Several previous observational studies have shown that abnormal sphingomyelin metabolism may be implicated in the pathogenesis of Alzheimer's disease. To determine the causal relationship between sphingolipid abundance and gut microbiota abundance at the genetic level, we conducted a Mendelian randomization (MR) investigation.

Methods We first used the TwoSampleMR and MRPRESSO packages for conducting two-sample MR studies. Second, we utilized random effect inverse variance weighting (IVW) as the principal method of analysis and used MR–Egger, the weighted median, the simple mode and the weighted mode as supplementary methods. Finally, we performed tests for heterogeneity and horizontal pleiotropy. These analyses were also conducted to evaluate the impact of individual SNPs on the outcomes of our analysis. A Bonferroni-corrected threshold of $p = 2.4e\text{-}4(0.05/211)$ was considered significant, and p values less than 0·05 were considered to be suggestive of an association.

Results The results showed that sphingolipid levels were suggestively associated with the abundance of 6 gut microbiota taxa. Specifically, two taxa were positively correlated with sphingolipid levels, including the family *Alcaligenaceae* ($p = 0.006$, OR 95% CI = 1.109 [1.030–1.194]) and the species *Ruminococcus callidus* ($p = 0.034$, OR 95% CI = 1.217 [1.015–1.460]). In contrast, negative correlations were observed with the abundances of 4 gut microbiota taxa, including the genus *Flavonifractor* ($p = 0.026$, OR 95% CI = 0.804 [0.663–0.974]), the genus *Streptococcus* ($p = 0.014$, OR 95% CI = 0.909 [0.842–0.981]), the species *Bacteroides caccae* ($p = 0.037$, OR 95% CI = 0.870 [0.763–0.992]), and the species *Haemophilus parainfluenzae* ($p = 0.006$, beta 95% CI = -0.269 [-0.462, -0.076]). The results presented a normal distribution, with no anomalous values, heterogeneity, or horizontal pleiotropic effects detected.

Conclusions This two-sample MR study revealed a potential causal relationship between sphingomyelin levels and gut microbiota abundance.

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Keywords Sphingomyelin, Alzheimer's disease, Gut microbiota abundance, Causality, Mendelian randomization

Introduction

Alzheimer's disease (AD) is a degenerative neurological condition resulting from injury to nerve cells (neurons) in the brain and represents the most prevalent form of dementia [1, 2]. Its pathological features mainly include abnormal forms of protein tau and β-amyloid plaques in neurons [2]. One study suggests that from 2000 to 2019, mortality rates associated with stroke, heart disease, and prostate cancer decreased, while the mortality rate for AD increased by approximately 145% during the same period. AD poses a serious threat to patients' quality of life, significantly impacting the workforce of families and society. A majority of AD patients exhibit atypical symptoms, particularly involving language, executive functioning, vision, behaviour and other impairments [3]. However, these people are often diagnosed very late. Although much research and progress has been made in the pathogenesis, diagnosis and treatment of AD, there are still many challenges. According to some published reports, sphingomyelin can be used as a target for AD treatment [4–8]. Sphingomyelin derived from progenitor cells is a special type of oligodendrocyte membrane [9]. Sphingomyelin is particularly important in the nervous system. It is a key component of neuronal myelin and contributes to the conduction of nerve signals. As a major regulator of axonal conduction in the central nervous system (CNS), it is essential for regulating motor, sensory and cognitive functions. Loss or alteration of sphingomyelin may affect the function of the nervous system, including aspects related to emotion, cognition and behaviour [10, 11]. Sphingomyelin is a key component of the myelin sheath or resident cell plasma membrane that regulates signal transduction, apoptosis, autophagy, senescence, necrosis and differentiation [12]. Sphingomyelin helps to maintain the integrity of the intestinal mucosa, thereby strengthening intestinal barrier function. This helps prevent harmful substances from entering the blood circulation, reduces inflammation and immune responses and maintains intestinal health [13]. Therefore, sphingomyelin plays an important role in supporting nervous system function, regulating the immune system, maintaining intestinal health and interacting with intestinal microorganisms.

The "brain-gut-microbiota axis" includes a wide range of communication networks between the brain, gut and microbiota [14]. Although the brain and intestine are two distant organs, they can play a very important role in the nervous system, immune system, and intestinal barrier and the influence of intestinal microorganisms through sphingomyelin [15].

Many recent studies have demonstrated a correlation between the gut microbiota and various ailments, including inflammatory bowel disease, Parkinson's disease, AD, type 2 diabetes, psoriasis, autism, anxiety, obesity, and schizophrenia [16, 17]. The majority of previous studies are case–control studies, and it was challenging to ascertain both the exposure and outcome. Furthermore, in prospective studies, the association between sphingomyelin abundance and gut microbiota abundance could be influenced by several confounding variables. These include age, environment, dietary habits, and lifestyle [18, 19]. Given that these factors are difficult to control for in observational studies, their effect on the association needs to be carefully considered. Therefore, causal inference between sphingomyelin and gut microbiota abundance is limited.

However, MR has emerged as a novel method for investigating the causal relationship between sphingolipid levels and gut microbiota abundance. MR employs genetic variation as an instrumental variable (IV) of exposure for estimating the causal correlation between exposure and disease outcome [20]. As the homozygous genotypes passed from parents to offspring are randomly assigned and any association between genetic variation and outcome remains unaffected by common confounding factors, the causal sequence is justifiable [21]. This approach has frequently been employed to investigate causal connections between various diseases. In this study, the Open Genome Wide Association Study (GWAS) database (https://gwas.mrcieu.ac.uk/) of the Medical Research Council Integrative Epidemiology Unit (MRC-IEU) was used to obtain summary data from the GWAS of sphingomyelin and gut microbiota abundance. Two-sample MR analysis was performed to assess the causal relationship between sphingomyelin levels and gut microbiota abundance.

Materials and methods

Study design

Our analyses used publicly available GWAS total statistical data. Each of the GWASs included in this study was ethically approved by their respective institutions. Based on these data, we used a two-sample MR design with sphingomyelin levels as the exposure and gut microbiota abundance as the outcome, revealing convincing evidence for a causal relationship between the two parameters. IVs were chosen using a strict set of inclusion and exclusion criteria, selecting only single nucleotide polymorphisms (SNPs) that were significantly correlated with sphingomyelin levels. Figure 1 provides an overview of the study.

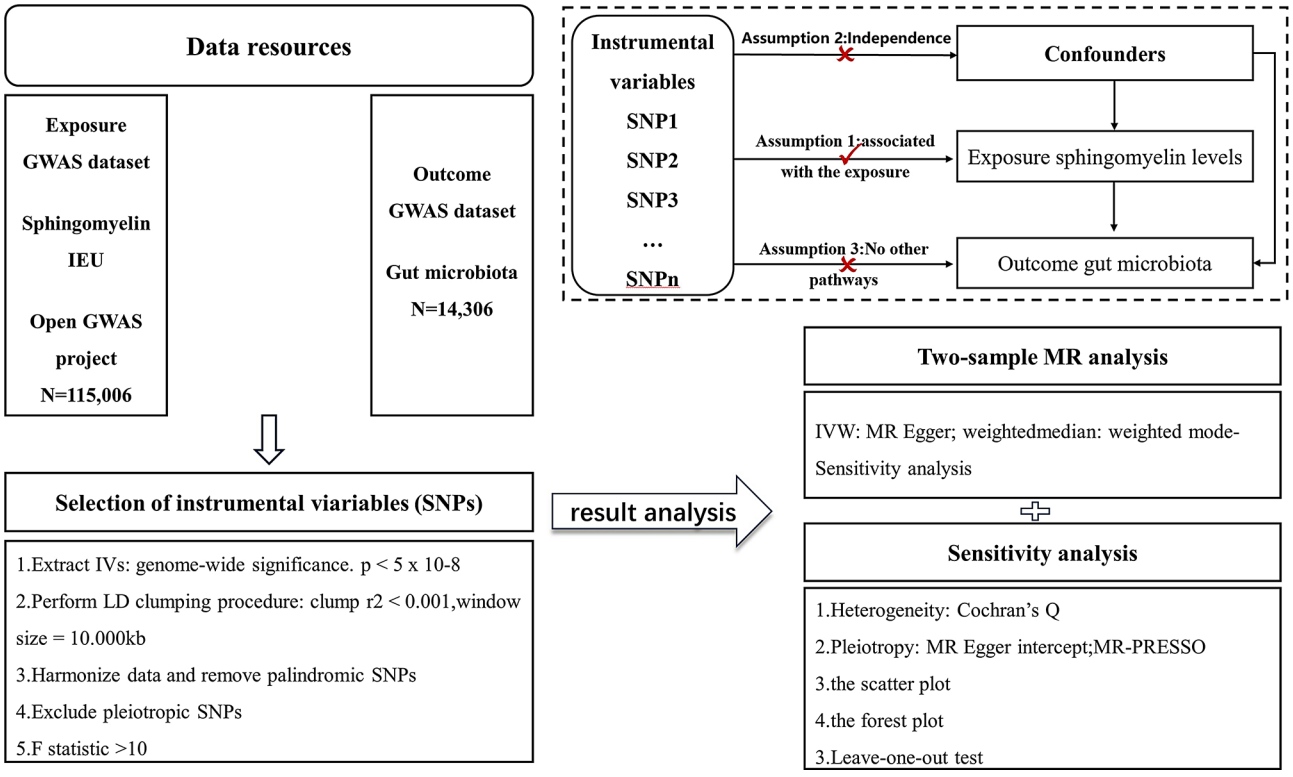

Fig. 1 The scheme of design and flowchart of this study

Data sources

All the data for this study were obtained from the MRC-IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/). One is related to the level of sphingomyelin [22], and the other is related to the abundance of the gut microbiota [23]. The GWAS data on sphingomyelin were all from blood samples of European races, and the GWAS provided strong evidence through MR, including 11,590,399 IVS from 115,006 UK biobank participants. And it was measured by targeted high-throughput NMR metabolomics from Nightingale Health in UK Biobank. During the analysis, sex, age, fasting status and genotyping batch were revised [24].

Information on gut microbiota abundance via GWAS was obtained through a previous meta-analysis that examined the relationship between genetic variation in human autosomes and gut microbiota abundance. The analysis incorporated sequencing profiles of 16 S ribosomal RNA genes and genotype data from 14,306 European individuals across 24 cohorts. The gut microbiota abundance in the initial study was classified into 257 taxa across six taxonomic levels: phylum [p], class [c], order [o], family [f] genus [g] and species [s]. Unknown taxa were excluded from the results. The study included 211 taxa from 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. Species-level taxa, which had ambiguous or unclear annotations, were excluded from the analysis, with only a small number of clearly annotated species retained, but these were not analyzed separately. The conditions for analysis were determined by employing a microbial quantitative trait locus (mbQTL) map to identify genetic variants that influence the relative abundance of microbial taxa. Several hypervariable regions (V3-V4, V1-V2, V4) within the 16 S ribosomal RNA (rRNA) gene serve as primary areas for examining the composition of the gut microbiota [23].

Instrument variable selection

The selection of IVS for analysis must be closely related to the exposure factors to ensure the soundness of the data and the precision of the outcomes. We performed quality checks on SNPs to obtain IVs that met three requirements: (1) The genetic variation associated with sphingomyelin levels, specifically SNPs, was obtained from the corresponding GWAS at the genome-wide significance level of $p<5\times10^{-8}$. Ensuring that there are no IVS with F values formula: $(R2/(R2-1))\times((N-K-1)/K)<10$ To ensure a robust correlation between IVS and exposure factors, it is necessary to consider certain factors. R2, which represents the exposure variance explained by the selected SNP, which represents the sample size, and k, which indicates the number of IVSs included) [25, 26]; (2) To test the MR hypothesis, we estimated the linkage disequilibrium between SNPs based on 1000 European genome

groups as a reference population. We used independent SNPs as IVSs (SNPs without linkage disequilibrium, $r^2 < 0.001$ and clumping window size = 10,000 kb); (3) We removed palindromic SNPs to prevent alleles from affecting the causal relationship between sphingomyelin levels and gut microbiota abundance. The selection of these key variables ensured the reliability of our research findings.

Mendelian randomization analysis

Fixed or random effects inverse variance weighted (IVW) methods, weighted median estimation model (WME) methods, MR–Egger regression and MR pleiotropic residual and outlier (MR-PRESSO) tests are well suited for estimating the potential causal link between sphingomyelin levels and gut microbiota abundance [27–29]. We used the IVW approach as the key analysis because it provided the most correct effect estimates, and almost all MR analyses used this approach as the primary analysis [30]. The IVW technique first uses the delta method and the Wald estimator to calculate the ratios of individual SNPs prior to consolidating the calculated estimates of each SNP to derive the primary causal estimates [31]. Cochran's Q test was utilized to analyse heterogeneity within the chosen SNPs. If there was heterogeneity ($p < 0.05$), the random effect IVW technique was applied; otherwise, the random effect or fixed effect IVW technique was selected [32, 33]. Since the outcomes of the IVW technique are susceptible to effective instruments and potential pleiotropy effects, a sensitivity analysis was executed to assess the strength of the correlation. First, we utilize the weighted median approach to estimate associations due to its greater reliability in providing causal effect estimates in the absence of effective tools. Even if up to 50% of the weight comes from invalid SNPs, WME can obtain robust results. Furthermore, the use of the MR-Egger reintroduction methodology can aid in evaluating causal associations and can test the potential level of pleiotropy. If the p value of the intersection does not exceed 0.05, there may be pleiotropy among the SNPs [34]. Third, we validated the outcomes of the IVW method using the MR-PRESSO approach, which can be used to detect and correct outliers as a whole to determine whether SNPs have possible outliers and obtain a corrected association knot after removing potential outliers [29].

Statistical analysis

MR estimation performance was assessed using a web-based calculation tool from Stephen Burgess. This study employed R software (version 4.3.1, R Statistical Computing Foundation, Vienna, Austria) and TwoSampleMR (version 0.5.6) to conduct this two-sample MR analysis to investigate the association between sphingolipid levels and gut microbiota abundance [35, 36]. In this study, five efficient MR analysis methods were employed: the inverse variance weighted (IVW) method, MR-Egger, weighted median, weighted mode, and simple mode methods. The IVW method serves as the primary MR analysis approach, with the other four methods typically utilized as supplementary methods [37, 38]. To address multiple testing, we applied a Bonferroni correction, setting the significance threshold to 2.4e-4 (i.e., 0.05/211, where 211 represents the number of putative risk factors). P-values between 2.4e-4 and 0.05 were considered suggestive of potential associations. The power of MR to detect causal effects depends on the proportion of variance in the risk factor explained by the genetic variants used as instruments. Therefore, we estimated the study power at an α of 0.05 for each risk factor a priori.

Results

Selection of IVs

Based on the criteria for selecting IVs, 66 SNPs were used as instruments. The F-statistic for all IVs exceeded 10, demonstrating that our chosen SNPs had a robust impact on IVs without any sign of weak IV bias. The features of all the SNPs are given in Table 1 of Supplementary File 1.

MR analysis

According to the IVW method, sphingomyelin levels were significantly associated with the abundance of 12 gut microbiota. Based on the hierarchical inclusion relationships among phylum, class, order, family, genus, and species, where a class is a subcategory of a phylum, we removed overlapping categories. For example, in the case of "Phylum *Proteobacteria*, Order *Burkholderiales*, Family *Alcaligenaceae*," we retained "Family *Alcaligenaceae*." Ultimately, we identified 6 gut microbiota abundances related to sphingomyelin levels. The findings indicated a positive correlation between the level of sphingomyelin and the family *Alcaligenaceae* ($p = 0.006$, OR 95% CI = 1.109 [1.030–1.194]) and the species *Ruminococcus callidus* ($p = 0.034$, OR 95% CI = 1.217 [1.015–1.460]). Fungal abundance was negatively correlated with genus *Flavonifractor* ($p = 0.026$, OR 95% CI = 0.804 [0.663–0.974]), genus *Streptococcus* ($p = 0.014$, OR 95% CI = 0.909 [0.842–0.981]), species *Bacteroides caccae* ($p = 0.037$, OR 95% CI = 0.870 [0.763–0.992]) and species *Haemophilus parainfluenzae* ($p = 0.006$, beta 95% CI = -0.269 [-0.462, -0.076]) (Table 1). Results on the traits of 211 Gut Microbiota Species are given in the Supplementary File 2. The MR data are presented in a scatter plot (Fig. 2), and the causal effect of sphingomyelin levels on the abundance of a single SNP in the gut microbiota is shown in the forest plot (Figs. 3 and 4).

The slope of the line indicates the causal relationship between the various MR techniques. Next, the MR–Egger and IVW tests were used to continue to examine

Table 1 MR assessed the association between sphingomyelin levels and gut microbiota

Exposure	Outcome	Nsnp	Method	OR	95%CI	*P*
Sphingomyelin levels	family Alcaligenaceae	50	MR Egger	1.223	1.056, 1.415	0.01
			Weighted median	1.125	1.003, 1.261	0.044
			Inverse variance weighted	1.109	1.030, 1.194	0.006
			Simple mode	1.095	0.890, 1.347	0.395
			Weighted mode	1.139	1.010, 1.285	0.04
	species Ruminococcus callidus	49	MR Egger	1.114	0.777, 1.597	0.561
			Weighted median	1.080	0.808, 1.443	0.605
			Inverse variance weighted	1.217	1.015, 1.460	0.034
			Simple mode	1.001	0.593, 1.690	0.996
			Weighted mode	1.001	0.720, 1.393	0.994
	genus Flavonifractor	50	MR Egger	0.697	0.476, 1.023	0.071
			Weighted median	0.807	0.598, 1.090	0.162
			Inverse variance weighted	0.804	0.663, 0.974	0.026
			Simple mode	0.917	0.536, 1.571	0.755
			Weighted mode	0.756	0.533, 1.073	0.124
	genus Streptococcus	50	MR Egger	0.947	0.813, 1.102	0.485
			Weighted median	0.962	0.857, 1.079	0.503
			Inverse variance weighted	0.909	0.842, 0.981	0.014
			Simple mode	0.947	0.765, 1.172	0.619
			Weighted mode	0.941	0.836, 1.060	0.322
	species Haemophilus parainfluenzae	52	MR Egger	0.875	0.593, 1.290	0.504
			Weighted median	0.829	0.626, 1.099	0.192
			Inverse variance weighted	0.764	0.630, 0.927	0.006
			Simple mode	0.931	0.569, 1.524	0.777
			Weighted mode	0.906	0.665, 1.233	0.533
	species Bacteroides caccae	51	MR Egger	0.827	0.635, 1.076	0.164
			Weighted median	0.890	0.742, 1.067	0.207
			Inverse variance weighted	0.870	0.763, 0.992	0.037
			Simple mode	0.957	0.689, 1.329	0.796
			Weighted mode	0.941	0.771, 1.149	0.555

the heterogeneity of the results, and all P values > 0.05 suggested that there was no heterogeneity in our results (Table 2). The Egger intercept was used to assess the horizontal pleiotropy between the IV, and the results showed no evidence of horizontal pleiotropy (Table 3). The results of the leave-one-out approach showed that certain individual SNPs could lead to deviations in terms of genetic prediction (Fig. 5). For the family *Alcaligenaceae*, we observed that two SNPs yielded significant Wald estimates. When these SNPs were excluded from the MR analyses, the MR estimate decreased but remained statistically significant, suggesting that these SNPs are robust contributors to the association. This finding indicates that the association between the genetic variant and the outcome is not solely driven by these SNPs but is also supported by other SNPs within the family. Regarding the species *Ruminococcus callidus*, one of the SNPs exhibited a significant Wald estimate that was in the opposite direction of the overall MR estimate. This discrepancy may suggest the presence of horizontal pleiotropy or other genetic confounders influencing the association. Furthermore, when four SNPs were excluded from the analysis, the MR estimate for these SNPs became non-significant. Using leave-one-out sensitivity analysis confirmed the stability of our study results when systematically removing individual SNPs. However, no significant outliers or horizontal pleiotropy ($P > 0.05$) were found in our MR analysis using MR-PRESSO (Table 4).

Discussion

The gut microbiota is a complex ecosystem. Maintaining the balance of gut microbiota abundance in the body is important because it can not only mediate the interaction between the human host and its environment but also mediate the balance between human health and disease [39]. Research on the gut microbiota is constantly developing. With the advancement of high-throughput sequencing technology, scholars have attained a more profound understanding of the variety and role of the gut microbiota. This research field has covered many aspects, including the role of microbes in neurodegenerative diseases (such as AD and PD) [40, 41]; cardiovascular diseases (such as hypertension, atherosclerosis, and heart failure) [42, 43]; metabolic diseases (such as obesity and

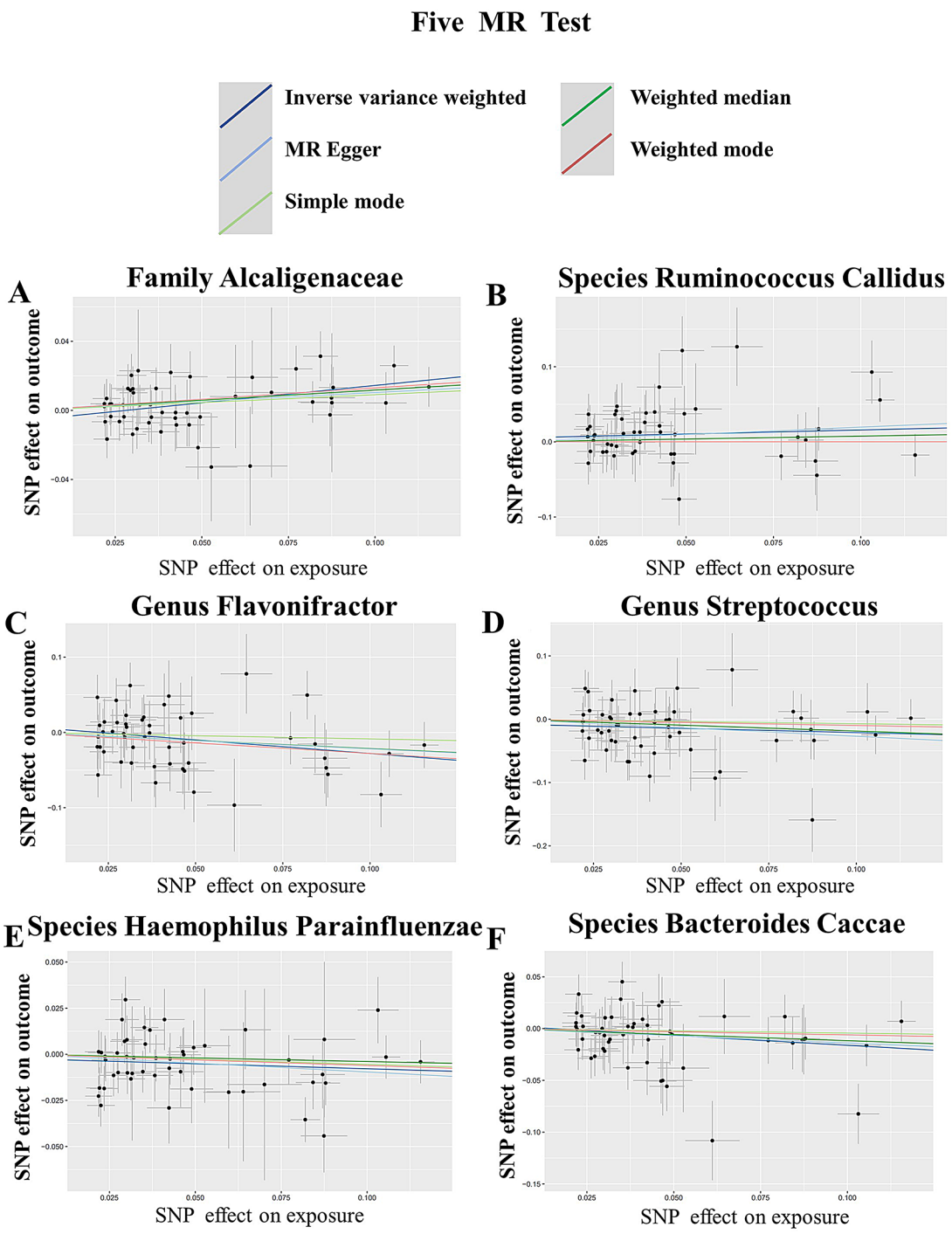

Fig. 2 Scatterplot of the results of MR analysis of the association between sphingomyelin levels and gut microbiota abundance. (**A**) family *Alcaligenaceae*, (**B**) species *Ruminococcus callidus*, (**C**) genus Flavonifractor, (**D**) genus *Streptococcus*, (**E**) species *Haemophilus parainfluenzae*, (**F**) species *Bacteroides caccae*. The gradient of the line indicates the causal relationship between the various MR techniques

diabetes) [44]; gastrointestinal diseases (such as inflammatory bowel disease) [45] and a variety of other cancers, including non-small cell lung cancer and hepatocellular carcinoma [46]. In addition to brain-gut interactions, there are many recent studies on probiotics and prebiotics and their application in personalized medicine [47]. Understanding the abundance of gut microbiota is crucial for comprehending human health and disease mechanisms and creating novel therapeutic approaches. This field will continue to make important breakthroughs in the fields of medicine and health.

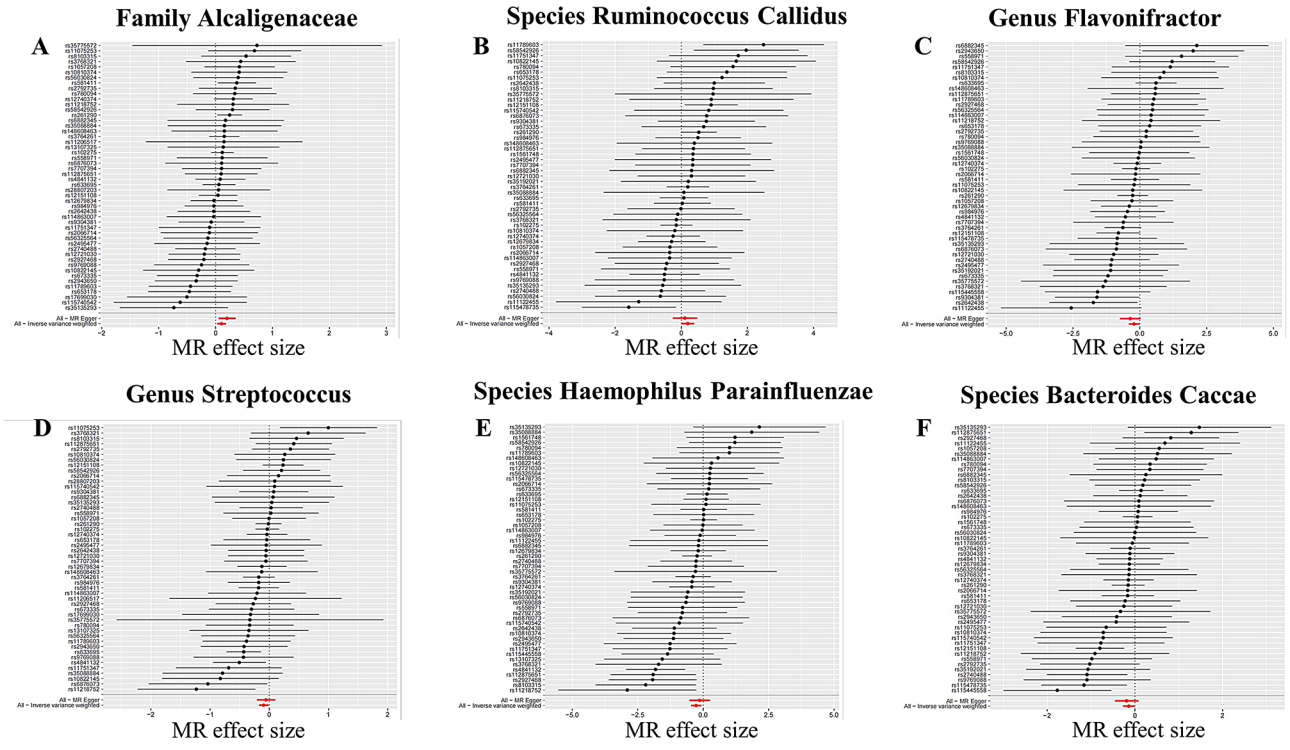

Fig. 3 The forest plot demonstrated the causal impact of sphingomyelin levels on the gut microbiota for a particular SNP. (**A**) family *Alcaligenaceae*; (**B**) species *Ruminococcus callidus*; (**C**) genus Flavonifractor; (**D**) genus *Streptococcus*; (**E**) species *Haemophilus parainfluenzae*; (**F**) species *Bacteroides caccae*

Outcome	Methond	OR(95% CI)	P−Value
family Alcaligenaceae	Inverse variance weighted	1.11(1.03 to 1.19)	0.006232769
species Ruminococcus callidus	Inverse variance weighted	1.22(1.01 to 1.46)	0.033874316
genus Flavonifractor	Inverse variance weighted	0.80(0.66 to 0.97)	0.026055481
species Haemophilus parainfluenzae	Inverse variance weighted	0.76(0.63 to 0.93)	0.006252313
genus Streptococcus	Inverse variance weighted	0.91(0.84 to 0.98)	0.014073485
species Bacteroides caccae	Inverse variance weighted	0.87(0.76 to 0.99)	0.037459961

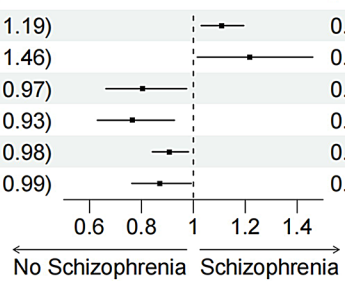

Fig. 4 The forest plot demonstrated the causal effects between sphingomyelin levels and gut microbiota abundance

An in-depth study of the gut microbiota revealed that the predictive potential of the gut microbiota is surprising. The gut microbiota may serve as a novel biomarker to predict the response to anti-PD-1 immunotherapy, as Jumin Huang and colleagues reported [48]. Xiaqing Yu, Wen Jiang, Russell Oliver Kosik and other researchers have shown that thyroid cancer can develop according to changes in intestinal microbial richness and diversity, and probiotics and prebiotics can regulate intestinal microecology to treat thyroid cancer [49]. Carmen Barrio et al. reviewed the associations between the gut microbiota and AD, PD, etc., and illustrated the benefits of supplementing probiotics and prebiotics for human cognition [50]. Therefore, based on the individual's gut microbiota abundance, clinicians can not only assess the individual's risk of developing diseases but also provide individualized dietary advice to promote gut health and overall health according to the composition of the patient's microorganisms to select and adjust the drug dose to improve the therapeutic effect of a disease. It is also possible to improve the microbial composition by predicting potential problems with an individual's gut microbiota abundance. Of course, researchers can also study and develop new treatments based on the role of gut microbes in diseases, such as fecal transplantation and microbiota interventions. Although the predictive potential of gut microbiota abundance is very promising, it still faces challenges, including our understanding of the causal relationship between microbes and health, standardized analysis methods and data privacy issues. However, this field is still developing rapidly and will

Table 2 Assessment of heterogeneity using different methods ($P > 0.05$)

Exposure	Outcome	Method	Cochran's Q	*P* value
Sphingomyelin levels	family Alcaligenaceae	MR Egger	31.035	0.973
		Inverse variance weighted	33.333	0.958
	species Ruminococcus callidus	MR Egger	46.432	0.496
		Inverse variance weighted	46.746	0.524
	genus Flavonifractor	MR Egger	47.854	0.479
		Inverse variance weighted	48.563	0.491
	genus Streptococcus	MR Egger	50.538	0.374
		Inverse variance weighted	50.94	0.397
	species Haemophilus parainfluenzae	MR Egger	52.466	0.379
		Inverse variance weighted	53.123	0.392
	species Bacteroides caccae	MR Egger	54.595	0.27
		Inverse variance weighted	54.81	0.297

Table 3 Directional horizontal Pleiotropy assessed by intercept term in MR Egger regression of the association betweensphingomyelin levels and gut microbiota abundance ($P > 0.05$)

Exposure	Outcome	Egger intercept	Se	*P* value
Sphingomyelin levels	family Alcaligenaceae	-0.006	0.004	0.136
	species Ruminococcus callidus	0.005	0.009	0.578
	genus Flavonifractor	0.008	0.010	0.404
	genus Streptococcus	-0.002	0.004	0.54
	species Haemophilus parainfluenzae	-0.008	0.010	0.432
	species Bacteroides caccae	0.003	0.007	0.662

continue to result in more innovations to personalized medicine and health management in the next few years.

To the best of our knowledge, this was the first MR study to assess whether there was a causal relationship between sphingomyelin levels and gut microbiota abundance. Genetic variations strongly associated with sphingomyelin levels were identified in the GWAS MRC-IEU OpenGWAS database. Based on genetic data from 115,006 Europeans, we found a causal relationship between sphingomyelin levels and the abundance of the six gut microbiota. Recent studies have shown that disorders of sphingomyelin metabolism are associated with AD, and the most commonly identified pathological events in AD are amyloid plaques and neurofibrillary tangles [51]. Moreover, abundant gut microbiota can secrete significant amounts of amyloid and lipopolysaccharide, which may contribute to the production of inflammatory cytokines associated with AD pathogenesis. *Bifidobacterium* species (probiotics) can enhance epithelial junctions, protect mucosal barrier function, protect intestinal mucosal integrity, reduce intestinal permeability, and reduce damage to the nervous system after inflammatory factors enter the blood circulation [52]. Harach and other researchers observed that with increasing *Bacteroidetes* abundance, the abundance of *Firmicutes* and *Proteobacteria* decreased significantly in AD mice and wild-type mice of the same age [53]. In addition, in a two-sample MR study, we were surprised to find that sphingomyelin levels were altered in the same way as gut microbiota abundance was. With increasing sphingomyelin levels, the abundances of two kinds of gut microbiota, the family *Alcaligenaceae* and the species *Ruminococcus callidus*, also increased. When the level of sphingomyelins decreased, the abundances of 4 gut microbiota, including species *Haemophilus parainfluenzae* and species *Bacteroides caccae*, increased. Species *Ruminococcus* are widespread gut bacteria that are ubiquitous in the human gut. It is capable of producing advantageous metabolites, including short-chain fatty acids, which may be advantageous to brain health. In individuals with AD, the abundances of *Ruminococcus callidus* is lower. These findings imply that *Ruminococcus callidus* could play a protective role in the development and progression of AD through the "gut microbiota abundance-gut-brain" connection [54–56]. Recent research has suggested a possible connection between specific types of *Streptococcus*, such as *Streptococcus pyogenes* and *Streptococcus suis*, and the development of AD. DNA from *Streptococcus* bacteria has been detected in the brain tissue of individuals infected with AD. These gut microbiota may invade the brain through various means, including the blood, causing inflammation and neuronal damage [57]. The genus *Flavonifractor* has also been found to be increased in elderly individuals with cognitive impairment [58].

In the study of Dong-oh Seo et al., regulating the gut microbiota appears as a promising strategy to slow the progression of AD. The abundance of gut microbiota directly reflects the diversity and quantity of microbes in the intestine. These microbes can influence the host through their metabolites, impacting the immune system and overall health. The ways in which gut microbiota affect host health are intricate, involving modulation of the immune system, synthesis of neurotransmitters, and production of metabolites [59–61]. Therefore, directly measuring microbiota abundance provides a comprehensive understanding of their potential effects on disease states. The interaction between gut microbiota and the brain is considered part of the gut-brain axis. Studies indicate that gut microbiota can influence neurological functions and inflammatory responses through metabolites such as short-chain fatty acids, which are relevant

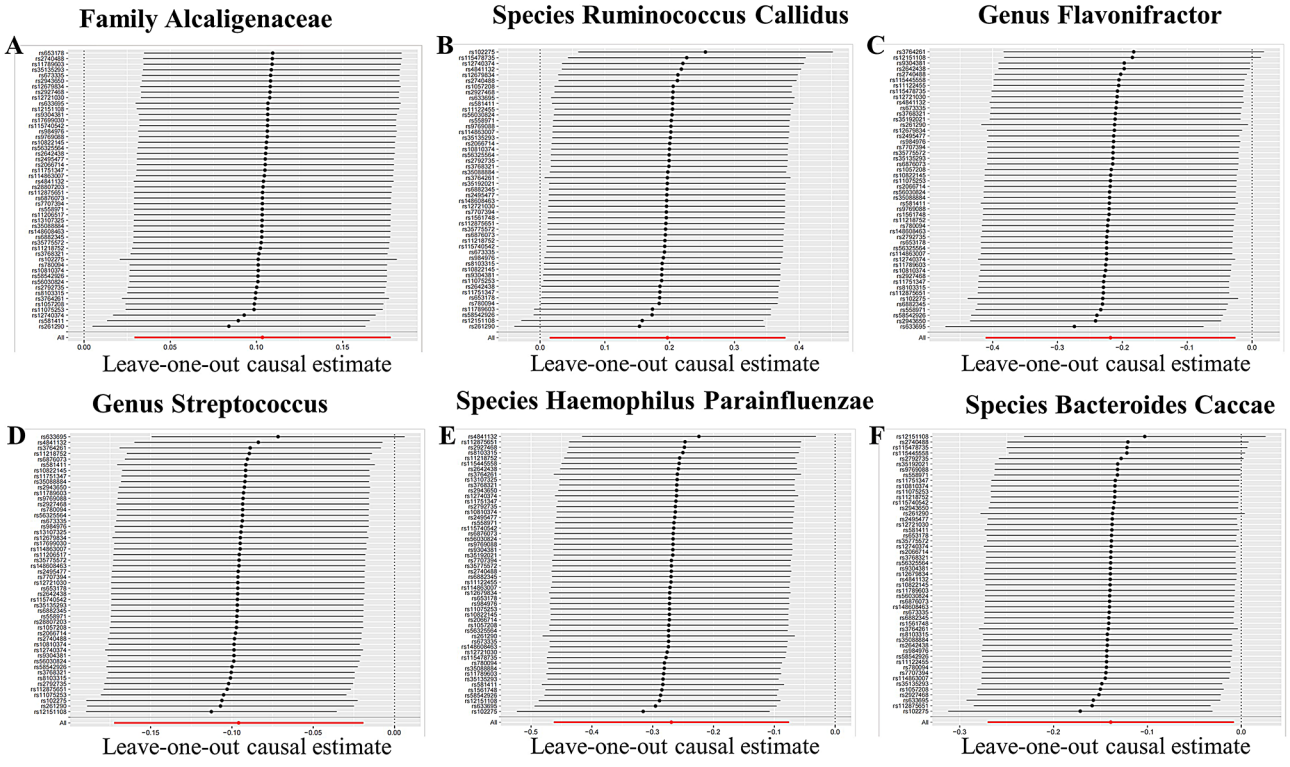

Fig. 5 Leave-one-out analysis about sphingomyelin levels and gut microbiota abundance. (**A**) family *Alcaligenaceae*; (**B**) species *Ruminococcus callidus*; (**C**) genus Flavonifractor; (**D**) genus *Streptococcus*; (**E**) species *Haemophilus parainfluenzae*; (**F**) species *Bacteroides caccae*

Table 4 MR-PRESSO analysis for the association between betweensphingomyelin levels and gut microbiota abundance. ($P > 0.05$)

Exposure	Outcome	MR Analysis	Causal estimate	SD	T	*P*-value	RSSobs	Global test *P*-value
Sphingomyelin levels	family Alcaligenaceae	MR-PRESSO	0.106	0.031	3.462	0.001	38.591	0.959
	species Ruminococcus callidus		0.189	0.090	2.086	0.421	51.210	0.499
	genus Flavonifractor		-0.204	0.961	-2.121	0.039	51.860	0.503
	genus Streptococcus		-0.942	0.038	-2.511	0.015	57.165	0.437
	species Haemophilus parainfluenzae		-0.249	0.010	-2.490	0.016	59.648	0.349
	species Bacteroides caccae		-0.142	0.067	-2.124	0.038	60.286	0.303

to AD pathogenesis. Modulating microbiota through dietary adjustments, probiotics, prebiotics, or fecal microbiota transplantation represents potential therapeutic avenues to improve symptoms and pathological processes in AD patients [62, 63]. Therefore, we can detect changes in sphingomyelin levels in the early stage by examining the gut microbiota for early diagnosis and treatment of AD.

However, Clinical trials investigating the potential therapeutic role of gut microbiota in AD are still in early stages. While preliminary research and animal models offer support, further large-scale, randomized controlled trials in humans are necessary to validate and assess efficacy. This includes deeper insights into the exact role of microbiota in AD pathogenesis and determining how specific microbial communities or metabolites impact disease progression and symptoms. Additionally, evaluating the long-term effectiveness and safety of different interventions, such as dietary modifications and probiotic therapies, remains crucial.

The key benefit of this study is the realization that there is a causal relationship between the level of sphingomyelin and the abundance of the gut microbiota at the gene level. The content of sphingomyelin can be determined by the abundances of these gut microbiota constituents for early diagnosis and treatment of AD. However, our study has several limitations. First, it should be noted that the GWAS summary data used in our study were only from European patients. In turn, this may have led to biased estimations and the potential lack of universal applicability. Second, our sample size may not be large enough. The more complete GWAS genome sequencing analysis data that can be obtained, the more precise and reliable the outcomes will be. Thirdly, MR analysis may

be influenced by various biases, so we employed multiple models to test the MR assumptions. Due to its biological plausibility and multi-stage statistical process, it may be overly conservative and could potentially miss gut microbiota abundances causally related to phospholipid levels when applying strict multiple testing corrections. Finally, although we found strong and suggestive evidence for an association between sphingolipid levels and the abundance of six gut microbiota abundance after applying Bonferroni correction to account for multiple testing, we cannot rule out the possibility that these findings may represent false positives. We only analyzed the genetic factors of phospholipid levels and the abundance of six gut microbiota abundance, so caution should be exercised in interpreting the results. Gut microbiota abundance can be influenced by various environmental factors such as dietary habits, exercise, or acquired health conditions, which generally have low heritability. Therefore, we acknowledge our inability to determine whether genetic tools are associated with these confounding factors.

Conclusions

In conclusion, we conducted an in-depth study of the causal relationship between the level of sphingolipids and the abundance of gut microbiota using two-sample MR. On the basis of the findings of previous studies and the present paper, we found that alterations in sphingomyelin can cause changes in the abundances of the six gut microbiota abundances. Therefore, we posit that such strains have the potential to serve as novel biomarkers, affording valuable insights into the prevention, early diagnosis and treatment of AD.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04207-3.

Supplementary Material 1

Supplementary Material 2

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Author contributions

J.Z.X: Conceptualization, Funding acquisition, Conceptualization, Supervision; L.P.W: Software, Investigation, Writing– original draft; Y.T: Investigation, Resources; Y.Y.D: Investigation, Methodology; M.Q.Y: Investigation, Visualization; Z.H.Y: Investigation, Resources; X.Y: Writing– review & editing.

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Data availability

All the data for this study were obtained from the MRC-IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/) [18]. The GWAS IDs for sphingomyelin levels and gut microbiota abundance are provided in Table 2 of Supplementary File 1. The code used for the Two-Sample MR analysis is publicly available and can be accessed at https://mrcieu.github.io/TwoSampleMR/index.html.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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