

## Research Report

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# Relationship Between Sphingomyelin and Risk of Alzheimer's Disease: A Bidirectional Mendelian Randomization Study

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### Abstract.

**Background:** Alzheimer's disease (AD) is a complex neurodegenerative disorder whose etiology involves multiple genetic and environmental factors. Sphingomyelin (SM) is a type of sphingolipid found in cell membranes, and recent evidence suggests a potential link between SM and AD. However, the nature of this relationship remains unclear.

**Objective:** To elucidate the potential causal relationship between SM levels and the risk of developing AD using a two-sample Mendelian randomization approach.

**Methods:** The study utilized data extracted from the genome wide association study database. The primary analysis method was the inverse variance weighted (IVW) method, which was supplemented by weighted median, weighted mode, and MR Egger methods. The study specifically investigated the bidirectional causal relationship between SM and AD, evaluating odds ratios (OR) with a 95% confidence interval (95%CI).

**Results:** Elevated levels of SM were found to be a risk factor for AD, as shown by IVW(MRE) [OR: 1.001, 95%CI: 1.000 to 1.002;  $p=0.020 < 0.05$ ], IVW(FE) [OR: 1.001, 95%CI: 1.001 to 1.002;  $p=3.36e-07 < 0.05$ ], and MR Egger. Conversely, AD was demonstrated to lead to an increase in SM levels [IVW(MRE): OR: 5.64e+08, 95%CI: 1.69e+05 to 1.89e+12;  $p=1.14e-06 < 0.05$ ], with consistent findings across the IVW(FE), MR Egger, weighted median, and weighted mode methods.

**Conclusions:** The study establishes a bidirectional positive correlation between SM and AD. Increased SM levels are associated with a higher risk of developing AD, and the presence of AD can further elevate SM levels, potentially exacerbating the disease's progression.

Keywords: Alzheimer's disease, bidirectional Mendelian randomization, genome-wide association study, relationship, sphingomyelin

## INTRODUCTION

Alzheimer's disease (AD) is a progressive central nervous system degenerative disorder characterized by learning and memory deficits, as well as a progressive decline in cognitive function [1]. With

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the gradual increase in human life expectancy and advancements in diagnostic methods, the incidence and total number of AD cases are rising each year. Consequently, AD has become one of the most significant threats to the quality of life and health of the elderly, posing a substantial challenge to global economic development [2]. AD belongs to cortical dementia and degenerative dementia and is an irreversible form of dementia. Clinically, the onset is insidious, characterized by a continuous and progressive decline in cognitive functions without remission. The hallmark of AD is cognitive impairment, with prominent memory disturbances. Additionally, it may manifest with aphasia, acalculia, apraxia, and agnosia, and lead to impairments in social, daily living, or occupational functions, exhibiting a unique neuropsychological deficit pattern. The clinical manifestations of AD include cognitive impairment symptoms, non-cognitive neuropsychiatric symptoms, and a decline in social and daily living functions. Among these, the decline in social and daily living functions is a consequence of cognitive impairment and non-cognitive neuropsychiatric symptoms, primarily characterized by reduced learning ability, working capacity, and self-care abilities [2, 3]. The primary pathological features of AD are the deposition of senile plaques formed by amyloid- $\beta$  ( $A\beta$ ) protein accumulation, neurofibrillary tangles resulting from hyperphosphorylation of tau protein, and neuron loss in specific brain regions [3]. At present, the etiology and pathogenesis of AD remain elusive, making accurate and effective treatments hard to find. Thus, there's an urgent need to explore strategies for preventing and treating AD.

Studies have indicated that lipids, especially cholesterol and sphingomyelin (SM), might play crucial roles in the pathogenesis of AD [4, 5]. SM is a vital lipid component involved in the structure and function of cell membranes. Essential for the normal function of the nervous system, it constitutes a significant part of the myelin sheath. Given that SM is enriched in the myelin sheath and closely related to cholesterol in lipid rafts, abnormalities in SM metabolism could lead to AD. Current evidence has identified a direct link between SM metabolic disturbances and neurodegenerative diseases in the brain [6], suggesting SM could be a potential breakthrough in AD research.

Current studies suggest a possible relationship between SM and AD. However, results remain inconsistent, and the precise causal relationship has been

debated, with contradictions across various studies. While some investigations have found increased SM levels in AD patients [7, 8], others have shown decreased SM levels [9–11] or no change at all [12]. Such disparities could be attributed to the varying sample sizes, research methodologies, regional and ethnic diversities, and other confounding factors of these studies.

The two-sample Mendelian randomization (TSMR) method is a powerful tool in epidemiological research. Its core concept revolves around using genetic variations as instrumental variables to determine causal relationships between risk factors and specific diseases [13–15]. TSMR can effectively circumvent confounding issues in observational studies, reverse causation, and challenges of representativeness and feasibility in randomized controlled trials [16]. To date, no studies, domestic or international, have utilized the TSMR method to evaluate the causal relationship between SM and AD. Therefore, to elucidate the causal relationship between SM and AD, this study employs the TSMR method to investigate the potential causal link between them, aiming to provide a theoretical basis for the prevention and treatment of AD.

## MATERIALS AND METHODS

### *Methods and research design*

In this study, we utilized publicly available genome wide association study (GWAS) summary databases and took SM and AD as the exposure or outcome factors respectively. We employed the TSMR method to assess the bidirectional causal relationship between SM and AD.

TSMR analysis requires satisfying three core assumptions: single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) must be closely associated with the exposure factor: 1) Select SNPs associated with SM that are genome-wide significant ( $p < 5E-08$ ). However, due to the limited number of SNPs that are genome-wide significant ( $p < 5E-08$ ) and related to AD, which are insufficient for TSMR analysis, we chose SNPs that are genome-wide significant ( $p < 5E-06$ ); 2) Remove SNPs with linkage disequilibrium ( $r^2 < 0.001$ , Clumping distance = 10,000 KB); 3) Exclude SNPs associated with confounding factors; 4) All SNPs must be screened through the calculation of the F-statistic to avoid weak instrument variable bias, with a value  $>10$  indicating the absence of weak

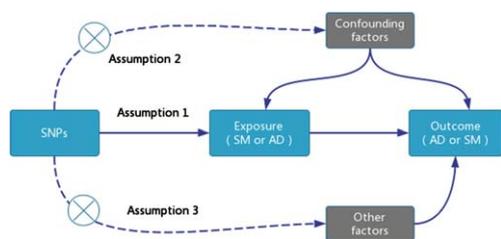


Fig. 1. TSMR Research Design Model.

instrument variables. The F-statistic is calculated as  $F = [(N - K - 1) / K] * [R^2 / (1 - R^2)]$ , where N is the sample size of the exposure factor, K is the number of instrumental variables, and  $R^2$  is the proportion of variance in the exposure factor explained by the instrumental variables [17] (Fig. 1).

Data source and selection of IVs

For this research, we accessed the GWAS database via the MR Base platform (<http://app.mrbase.org/>). Data concerning SM was derived from a study of 114,999 individuals, whereas the data for AD came from the UK Biobank, encompassing 488,285 Europeans and 12,321,875 SNPs (Table 1).

To align with the TSMR research design, IVs must be closely associated with the exposure factors SM or AD. We adopted a genome-wide significance threshold ( $p < 5E-08$ ) to select SNPs significantly associated with SM or AD. Using clumping procedures ( $r^2 < 0.001$ , Clumping distance = 10,000 KB), we removed SNPs that exhibited linkage disequilibrium. Ultimately, in the outcome data AD or SM corresponding to the exposure factors SM or AD, 59 independent SNPs related to the exposure factor SM were extracted as IVs (Fig. 2). However, only 4 independent SNPs related to the exposure factor AD were retrieved. Thus, we resorted to a substitute significance threshold of  $p < 5E-06$ . Under this criterion, 26 independent SNPs associated with the exposure factor AD were extracted as IVs (Table 2).

Moreover, we employed the F-test to evaluate whether the chosen IVs have a weak instrument bias. An F value greater than 10 indicates the absence of weak instrument bias, further corroborating the associativity hypothesis. The formula is  $F = [(N - K - 1) / K] * [R^2 / (1 - R^2)]$ , where N represents the sample size of the exposure factor, K is the number of instrumental variables, and  $R^2$  is the proportion of exposure variation explained by the IVs [18].

TSMR statistical analysis

This study primarily employed the inverse variance weighted (IVW) method for analysis. We used Cochran’s Q to test for heterogeneity. If  $p < 0.05$ , suggesting the presence of heterogeneity, we then utilized the multiplicative random effects (MRE) method to mitigate bias arising from heterogeneity. If  $p > 0.05$ , indicating no evident heterogeneity affecting our results, the fixed effects (FE) method was adopted for analysis [19, 20].

We also employed supplementary analysis methods such as weighted median, weighted mode, and MR Egger for validation. The MR Egger method is a way to detect and adjust for pleiotropy in TSMR analysis to obtain causal effect estimates. This tests whether the results are influenced by horizontal pleiotropy; if  $p > 0.05$ , then there is no horizontal pleiotropy, meaning IVs affect the outcome entirely through the exposure. If  $p < 0.05$ , suggesting horizontal pleiotropy exists, the MR Egger method is primarily used for analysis [21, 22]. The weighted median and weighted mode methods use a majority of IVs to determine the existence of a causal relationship; when a few IVs do not satisfy the validity assumption, the estimate remains robust, although not unbiased [23]. If the result from the IVW method is significant ( $p < 0.05$ ) and no pleiotropy is detected, even if the outcomes from other methods are not significant, as long as the direction of other methods is consistent, it can be considered a positive result [24].

To interpret the results more intuitively, this study employed the odds ratio (OR) and its 95% confi-

Table 1  
Summary of Genetic Data Information

variable	GWAS ID	Sample size	SNPs	Population	Sex	Year
SM	met-d-Sphingomyelins	114999	12321875	European	Males and Females	2020
AD	ieu-b-5067	488285	12321875	European	Males and Females	2022

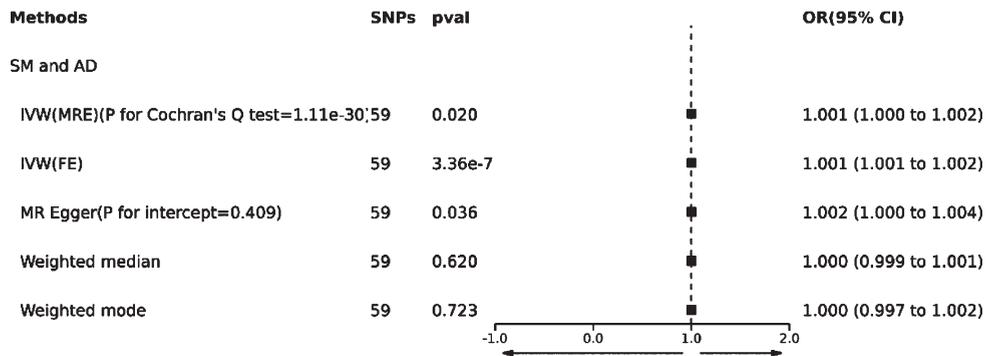


Fig. 2. TSMR analysis results of causal relationship between SM and AD.

Table 2  
TSMR analysis results of the causal relationship between AD and SM

Methods	SNPs	OR (95% CI)	p for association	p for Cochran's Q test	p for MR Egger intercept
AD and SM					
IVW(MRE)	26	5.64e+08 (1.69e+05 to 1.89e+12)	1.14e-06	4.36e-58	
IVW(FE)	26	5.64e+08 (6.35e+07 to 5.01e+09)	4.11e-73		
MR Egger	26	2.14e+11 (3.19e+05 to 1.44e+17)	8.46e-04		0.287
Weighted median	26	3.43e+07 (1.39e+04 to 8.44e+10)	1.34e-05		
Weighted mode	26	8.24e+12 (1.55e+11 to 4.38e+14)	8.66e-14		

dence interval (95% CI) as effect indicators. A  $p$  value less than 0.05 was considered statistically significant. Additionally, to ensure the overall effect's reliability, we conducted a "leave-one-out" analysis. By sequentially omitting one SNP and calculating the MR effect of the remaining SNPs, we assessed the potential outlier instrumental variables, thus gauging the stability of the TSMR analysis results [25].

## RESULTS

### Causal relationship results between SM and AD

Based on the methodology of this study, 59 SNPs related to SM were extracted as IVs. The effect of each SNP locus on AD through TSMR analysis is illustrated in Fig. 3A. All SNPs underwent the F-test, with F-values ranging from 29.777 to 2598.546, all  $>10$ , suggesting no weak instrument bias affecting the study's outcome, thus confirming the reliability of the data results. The MR Egger horizontal pleiotropy test ( $p$  for intercept = 0.409  $>0.05$ ) is shown in Fig. 2, indicating no horizontal pleiotropy. The funnel plot also demonstrates no apparent bias (Fig. 3B). Calculating Cochran's Q value ( $p = 1.11e-30 < 0.05$ ) indicates heterogeneity, leading the primary analysis method to be IVW(MRE) as seen in Fig. 2.

This study reveals a positive correlation between SM and AD, suggesting that SM might be a risk factor for the onset of AD [IVW(MRE): OR: 1.001, 95% CI: 1.000 to 1.002;  $p = 0.020 < 0.05$ ]. Concurrently, [IVW(FE): OR: 1.001, 95% CI: 1.001 to 1.002;  $p = 3.36e-07 < 0.05$ ] and the MR Egger method [OR: 1.002, 95% CI: 1.000 to 1.004;  $p = 0.036 < 0.05$ ] further validated this finding. However, neither the Weighted median nor Weighted mode analysis discovered similar outcomes (Figs. 2 and 3C).

Additionally, a "leave-one-out" analysis was performed. This step-by-step exclusion test suggested that no single SNP significantly influenced the result's robustness, ensuring the study's stability and reliability (Fig. 3D).

### Causal relationship results between AD and SM

For the reverse TSMR, 26 SNPs related to AD were extracted as IVs. The effect of each SNP locus on SM through TSMR analysis is shown in Fig. 4A. All SNPs underwent the F-test, with F-values ranging from 20.773 to 797.552, all  $>10$ , indicating no weak instrument bias affecting the results, affirming the reliability of the data. The MR Egger horizontal pleiotropy test ( $p$  for intercept = 0.287  $>0.05$ )

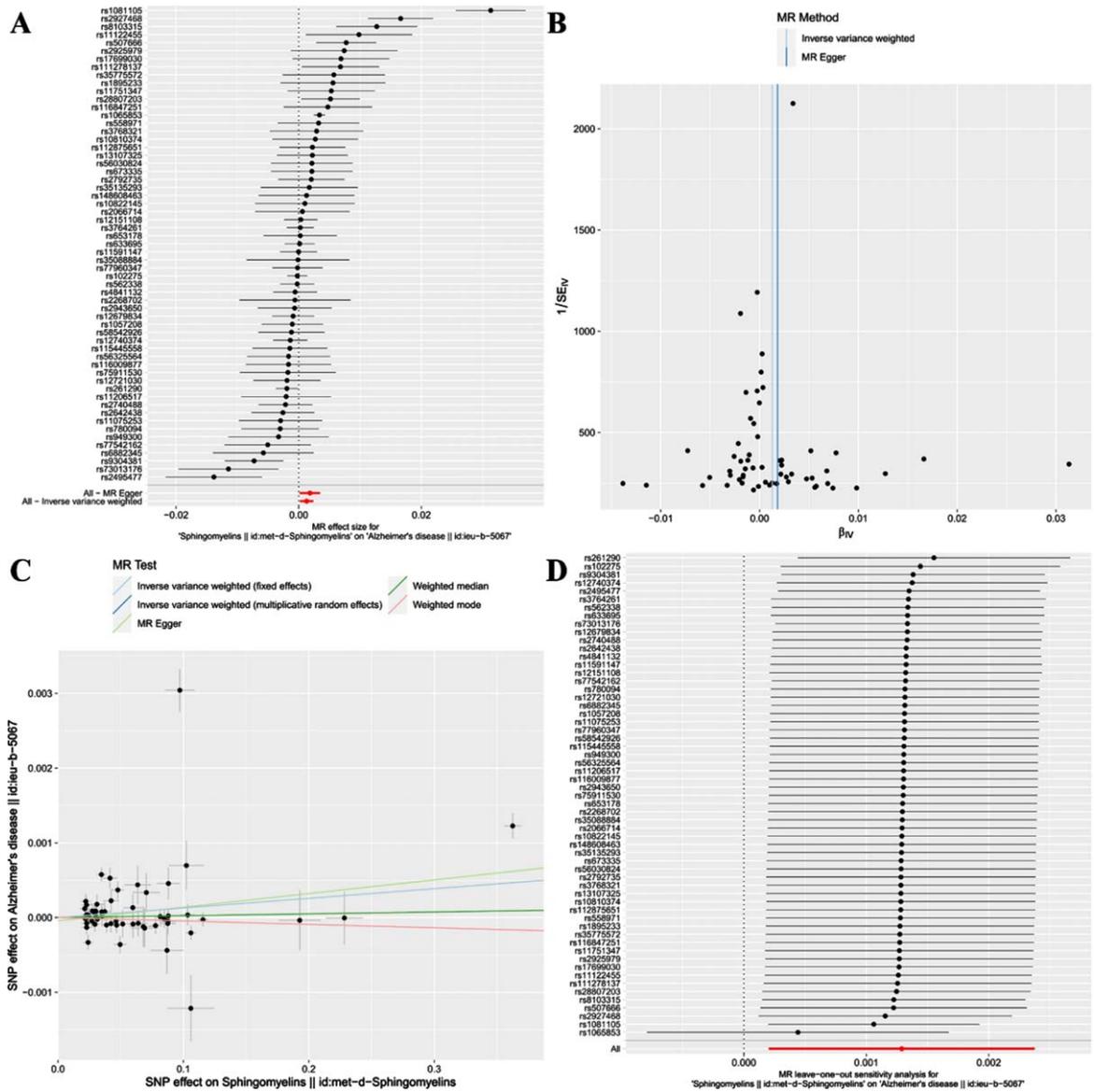


Fig. 3. Forest plot (A), funnel plot (B), scatter plot (C), and leave-one-out plot (D) illustrating the causal relationship between SM and AD.

is provided in Table 2, suggesting no horizontal pleiotropy; the funnel plot also indicates no apparent bias (Fig. 4B). Calculating Cochran's Q value ( $p = 4.36e-58 < 0.05$ ) shows heterogeneity, so the primary analysis method is IVW(MRE), as described in Table 2.

This study discovered a significant positive correlation between AD and SM, suggesting that AD could likely lead to an increase in SM [IVW(MRE): OR: 5.64e+08, 95% CI: 1.69e+05 to 1.89e+12;  $p = 1.14e-06 < 0.05$ ]. Concurrently, results from IVW(FE) [OR: 5.64e+08, 95% CI: 6.35e+07

to 5.01e+09;  $p = 4.11e-73 < 0.05$ ], MR Egger [OR: 2.14e+11, 95% CI: 3.19e+05 to 1.44e+17;  $p = 8.46e-04 < 0.05$ ], Weighted median [OR: 3.43e+07, 95% CI: 1.39e+04 to 8.44e+10;  $p = 1.34e-05 < 0.05$ ], and Weighted mode [OR: 8.24e+12, 95% CI: 1.55e+11 to 4.38e+14;  $p = 8.66e-14 < 0.05$ ] further confirm this finding (Table 2, Fig. 4C).

Additionally, after conducting the "leave-one-out" analysis and progressively excluding SNPs, the results indicate no single SNP significantly influences the robustness of the results, ensuring the study's stability and reliability (Fig. 4D).

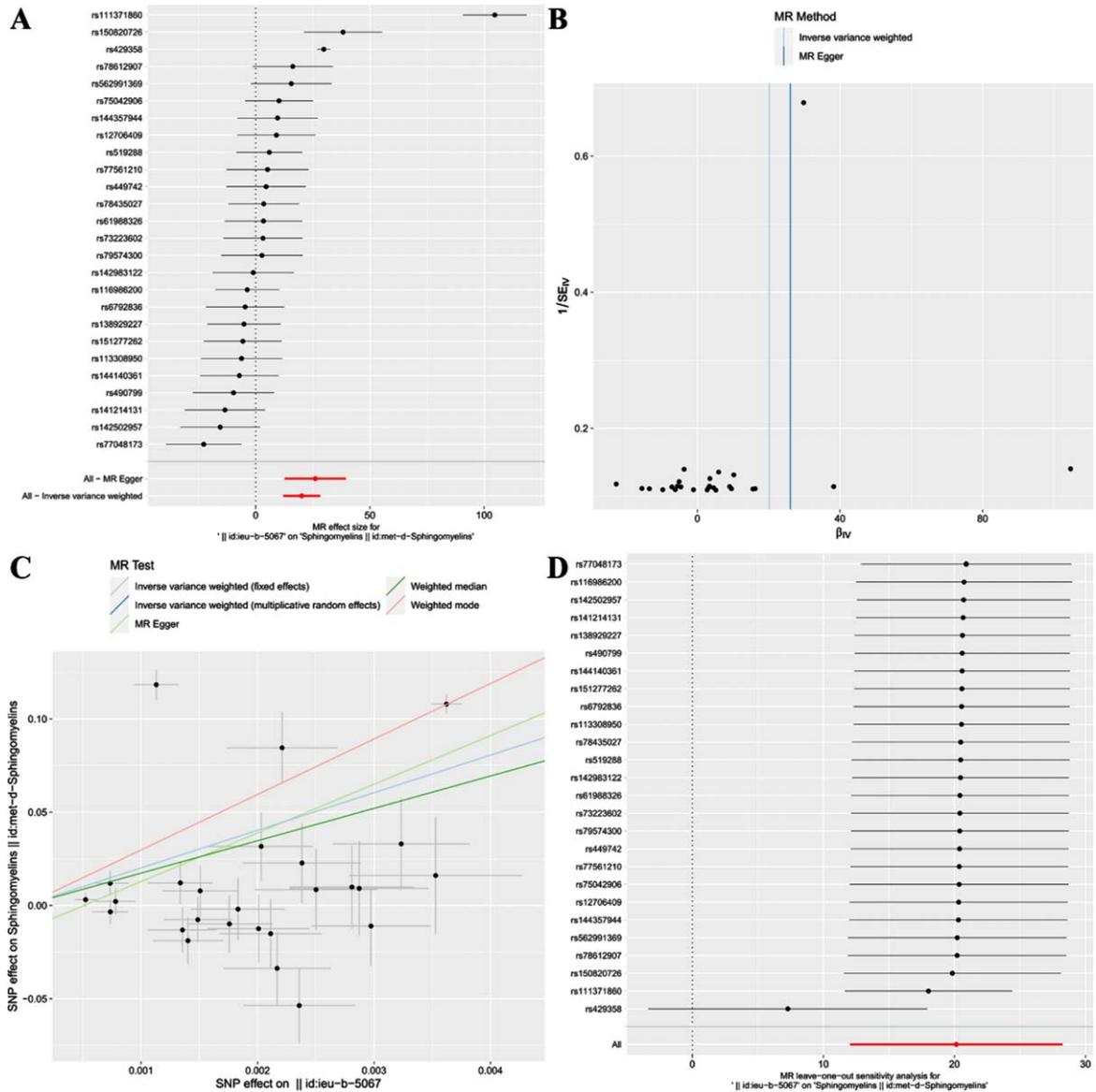


Fig. 4. Forest plot (A), funnel plot (B), scatter plot (C), and leave-one-out plot (D) illustrating the causal relationship between AD and SM.

**DISCUSSION**

This study employed a bidirectional TSMR research method to systematically assess the causal relationship between SM and AD. Predictions at the genetic level suggest a bidirectional positive correlation between SM and AD. On one hand, this indicates that SM is a risk factor for the onset of AD and is associated with an increased risk of AD. On the other hand, it suggests that AD may lead to a further increase in SM.

Our findings are consistent with previous observational studies that identify SM as a risk factor for the onset of AD, with higher concentrations of SM increasing the risk of developing AD. A metabolomic analysis of the AD brain revealed that high concentrations of SM are closely associated with increased severity of AD pathology and cognitive abnormality risks [26]. Depp et al. [27] used models of chronic myelin damage to systematically unveil its role in promoting amyloid protein deposition, a hallmark pathology of AD. The study revealed that myelin

damage likely triggers accelerated A $\beta$  production from axonal swelling, and diverts the attention of microglial cells, thus slowing down A $\beta$  clearance. This disrupts the brain's internal balance and accelerates plaque production. SM, being the most abundant sphingolipid in the brain and highly concentrated in myelin, may increase in concentration due to myelin damage. A 31P nuclear magnetic resonance study found increased SM in AD subjects' brain tissues, suggesting abnormal SM metabolism could lead to AD [28]. Thus, SM might serve as a biomarker for early detection and prevention of AD, and correcting SM metabolic disturbances could be a promising and innovative AD therapeutic strategy [26].

Concurrently, our reverse TSMR analysis found that AD leads to an increase in SM. Although current research presents mixed results regarding SM levels in AD patients, our study at the genetic level supports that AD causes an increase in SM levels. Kosicek et al. [29] speculate that the rise in SM levels in AD might be a cellular response to high ceramide levels, which then partially metabolizes into SM. Moreover, Agarwal et al. [30] found a close relationship between ABCA7 rs3764650 gene mutations and impaired SM transport function. The progression and severity of AD might be linked to ABCA7's impaired SM transport function. Cuyvers et al. [31] found that knocking out ABCA7 in mice significantly elevates both SM and ceramide levels. Under physiological conditions in normal populations, ABCA7 protein transports SM inside neuronal cells to APOA1/HDL, regulating neuronal ceramide metabolism and maintaining normal exosome production and secretion. SM-rich APOA1/HDL increases the affinity for soluble A $\beta$ , facilitating A $\beta$  clearance through the blood-brain barrier [32]. AD patients might impair ABCA7's SM transport function, resulting in increased SM levels.

Differences in past study outcomes could be attributed to factors like sample size, research methodology, regional and ethnic diversity, etc. Genetic variations remain stable throughout an individual's life, and allele classification and fixation are random. Therefore, the results discovered at the genetic level in this bidirectional TSMR study can avoid biases caused by various confounding factors.

However, our study has some limitations. Firstly, we used a genome-wide significance threshold ( $p < 5E-08$ ) and extracted only four independent SNPs related to the exposure factor AD. Choosing a limited number of SNPs as IVs might only explain a small part of the exposure variations, affecting the causal estimate's statistical power. Hence, we

adopted a significance threshold of  $p < 5E-06$  and extracted 26 independent SNPs related to the exposure factor AD as IVs. Secondly, TSMR analysis data was sourced from Europeans, and it remains to be verified if genetic differences exist across different ethnicities, countries, and regions. Lastly, although we have confirmed the bidirectional causal relationship between SM and AD, the underlying mechanism remains unclear. Further in-depth research is required to explore the interaction mechanism between SM and AD.

### Conclusion

In summary, through bidirectional TSMR analysis, this study found a bidirectional positive correlation between SM and AD. On one hand, it indicates that SM is a risk factor for the onset of AD, with increased SM being associated with a higher risk of AD onset. This study innovatively uses TSMR analysis to discover from a genetic perspective that there is a bidirectional positive correlation between SM and AD. On one hand, it suggests that SM is a risk factor for the onset of AD, with elevated levels of SM being associated with a higher risk of AD onset. On the other hand, it indicates that AD can also lead to a further increase in SM, potentially exacerbating the progression of AD. This study not only fills a gap in this field but also has positive clinical implications. We should advocate for enhanced monitoring of SM in clinical settings, providing new clinical evidence for the prevention and treatment of AD. Furthermore, it can also provide theoretical references for the development of new drugs, paving the way for the development of medications aimed at regulating SM in AD patients.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to report.

## DATA AVAILABILITY

The dataset generated during and analyzed during the current study are available from the MR Base database (<http://www.mrbase.org/>).

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