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Analysis of core target genes of vagus nerve stimulation in epilepsy, ischemic stroke, and depression

Separate insights from network pharmacology, bioinformatics, and Mendelian randomization

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Abstract

Epilepsy, ischemic stroke, and depression, as common neurological diseases, pose a serious threat to human health. Although vagus nerve stimulation (VNS) has been applied in the treatment of these diseases, its exact mechanism of action remains unclear. This study aims to deeply analyze the relationships between the core target genes of VNS and epilepsy, ischemic stroke, and depression by integrating network pharmacology, bioinformatics, and Mendelian randomization. The results showed that, compared with the control group, 159 differentially expressed genes were identified in the VNS-treated group. These genes were involved in biological processes such as "protein-RNA complex organization" and are enriched in metabolism-related pathways such as the "AMPK signaling pathway." There were 73, 54, and 108 overlapping genes between VNS-related genes and genes related to epilepsy, ischemic stroke, and depression, respectively. Through network analysis and Mendelian randomization analysis, several core genes that may play a protective role in the corresponding diseases were identified, such as *CPT1A*, *SUCLG1*, *IMMT*, *IVD*, and *PSMA2*. This study has revealed the potential molecular mechanisms possibly involved in VNS treatment of these neurological diseases. The findings offer crucial clues for further in-depth study of VNS treatment mechanisms, and may boost the development of more precise and effective treatment strategies.

Abbreviations: 95% CI = 95% confidence interval, DEGs = differentially expressed genes, GWAS = genome-wide association study, IS = ischemic stroke, IVs = instrumental variables, IVW = inverse variance weighted, MNC = Neighborhood Component Centrality, MR = Mendelian randomization, OR = odds ratio, PPI = protein–protein interaction, SNPs = single nucleotide polymorphisms, STRING = Search Tool for Recurring Instances of Neighbouring Genes, VNS = vagus nerve stimulation.

Keywords: depression, epilepsy, ischemic stroke, Mendelian randomization, vagus nerve stimulation

1. Introduction

Epilepsy, ischemic stroke (IS), and depression, as common neurological disorders, pose a serious threat to human health. Epilepsy is a chronic brain disorder characterized by sudden, abnormal electrical discharges of neurons in the brain, leading to brief episodes of disrupted brain function.[1] The disease can occur at all ages, especially among children and the elderly. Seizures not only cause physical suffering to patients, but frequent episodes may also affect their cognitive function, mental health, and quality of life, leading to numerous difficulties in aspects such as learning, work, and social interaction.[2,3]

IS is a severe medical emergency that cannot be predicted in advance and can exert long-lasting impacts on patients, their

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families, and communities. Each year, approximately 795,000 people are affected by IS. This not only inflicts a heavy toll on patients and their families but also burdens society significantly.[4,5] IS ranks among the leading causes of death and disability worldwide, placing a substantial financial stress on communities.[6]

Depression is a common mental disorder, with persistent low mood, decreased interest, self-blame, and self-guilt as its main clinical manifestations. Globally, it is estimated that more than 300 million people suffer from depression, accounting for 4.4% of the world's population.[7] Major depressive disorder not only involves abnormalities in emotions and mood but also leads to neurovegetative dysfunctions, such as affecting appetite and causing sleep disorders. It also brings about cognitive abnormalities, like inappropriate feelings of guilt and worthlessness, as well as psychomotor activity abnormalities, for example, agitation or retardation. It is one of the oldest and widely recognized medical disorders, which was clearly described in medical texts as early as in ancient Greece.[8]

Vagus nerve stimulation (VNS) is a method in which an implanted stimulator regularly delivers electrical pulses to stimulate the vagus nerve. This, in turn, regulates the release of neurotransmitters, neural plasticity, and the function of neural networks, ultimately improving disease symptoms.[9] It can project to other brainstem nuclei such as the locus coeruleus and raphe magnus via the nucleus of the solitary tract, and these brainstem nuclei, in turn, project extensively to the cerebral cortex, thus influencing the activities of the cerebral cortex.[10] As an important neural regulation therapy, VNS has been applied in the treatment of diseases such as epilepsy and depression and has demonstrated certain efficacy.[10,11] VNS can regulate depression by means of regulating the brain circuits mediating depressive symptoms through bottom-up stimulation, promoting neuroplasticity, altering neuronal firing patterns, reducing neuroinflammation, and modulating the gut microbiota–brain axis.[12] Previous literature has focused on the long-term efficacy and safety of VNS in treatment-resistant depression. The core conclusion is that VNS provides sustained therapeutic benefits to severe treatment-resistant depression patients who have not responded to multiple antidepressant treatments.[12] With limited safety risks, it represents a viable long-term treatment option.[13] VNS was U.S. Food and Drug Administration-approved for the management of drug-resistant epilepsy.[14] VNS exerts its effect through the vagal afferent network, regulating the thalamocortical circuits, which leads to a reduction in seizures in approximately 50% of patients. Meanwhile, research indicates that paired VNS is an effective treatment option that can bring long-term benefits to patients with upper-extremity limitations following IS.[15] However, despite certain achievements of VNS in clinical applications, its exact mechanism of action remains incompletely understood. In-depth research on the core target genes of VNS is crucial for uncovering the potential molecular mechanisms underlying its treatment of these diseases, and it is expected to provide a theoretical basis for the development of more effective treatment strategies.

Network pharmacology is an emerging discipline based on the theory of systems biology. It analyzes molecular networks in biological systems, such as protein–protein interaction (PPI) networks and metabolic networks, to explore the interaction relationships between drugs and disease-related targets. By constructing drug–target–disease networks, network pharmacology can comprehensively uncover potential therapeutic targets and related signaling pathways, providing a systematic and comprehensive perspective for understanding the mechanisms of drug action.[16,17] For example, in the study of the mechanisms of traditional Chinese medicine compound prescriptions, network pharmacology has successfully revealed multiple key targets and signaling pathways, providing new ideas for drug development and disease treatment.[18–21]

Bioinformatics integrates mathematical, statistical, and computer science methods to analyze and interpret biological data. It can screen out key disease-related genes from vast amounts of genetic data, analyze gene functions and the signaling pathways they participate in, providing crucial clues for studying the pathogenesis of diseases.[22]

Mendelian randomization (MR) is a method that uses genetic variations as instrumental variables (IVs) to infer the causal relationship between exposure and outcome. It enables more accurate inference of the causal relationship between genes and diseases.[23,24]

This study aims to integrate network pharmacology, bioinformatics, and MR methods to deeply analyze the relationships between the core targets of VNS and each of the 3 diseases: epilepsy, IS, and depression. We chose these 3 diseases for our research mainly based on the following considerations. First, all 3 are common neurological diseases that seriously affect human health. Second, the application of VNS in these 3 diseases has been approved by the U.S. Food and Drug Administration and has shown certain efficacy, yet the specific mechanisms remain unclear. In addition, the gene-related data of these diseases are relatively abundant, which makes it possible to integrate network pharmacology, bioinformatics, and MR research methods for our study. We hypothesize that VNS exerts its therapeutic effects by regulating specific core target genes, thereby influencing the biological processes and signaling pathways related to these diseases. This study will provide a new perspective for revealing the molecular mechanisms of VNS in treating neurological diseases. Figure 1 presents the framework diagram of this paper.

2. Materials and methods

2.1. Data source

The GSE210043 dataset in the Gene Expression Omnibus database collected peripheral blood mononuclear cells isolated from 4 epilepsy patients in the VNS treatment group and 4 epilepsy patients in the control group. Peripheral blood mononuclear cells were isolated for RNA sequencing. We used this dataset to obtain VNS-related targets. Genes related to epilepsy, IS, and depression were obtained from the GeneCards database, respectively. Genome-wide association study (GWAS) data for epilepsy and depression were obtained from FinnGen, and GWAS data for IS were obtained from the GWAS database (https://gwas.mrcieu.ac.uk/).

2.2. Differential expression analysis

The "limma" package in R software (R Core Team and the R Foundation for Statistical Computing) was used to conduct a differential analysis between the VNS treatment group and the control group in the GSE210043 dataset. R software is a global collaborative open-source project, with developers and contributors from all over the world. The screening criteria were log2-fold change > 1 and P value < .05, and differentially expressed genes (DEGs) were obtained accordingly.

2.3. Functional enrichment analysis

The "clusterProfiler" package in R software was used to perform Gene Ontology Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes enrichment analyses on the DEGs. The filtering criterion was set as $P < .05$.

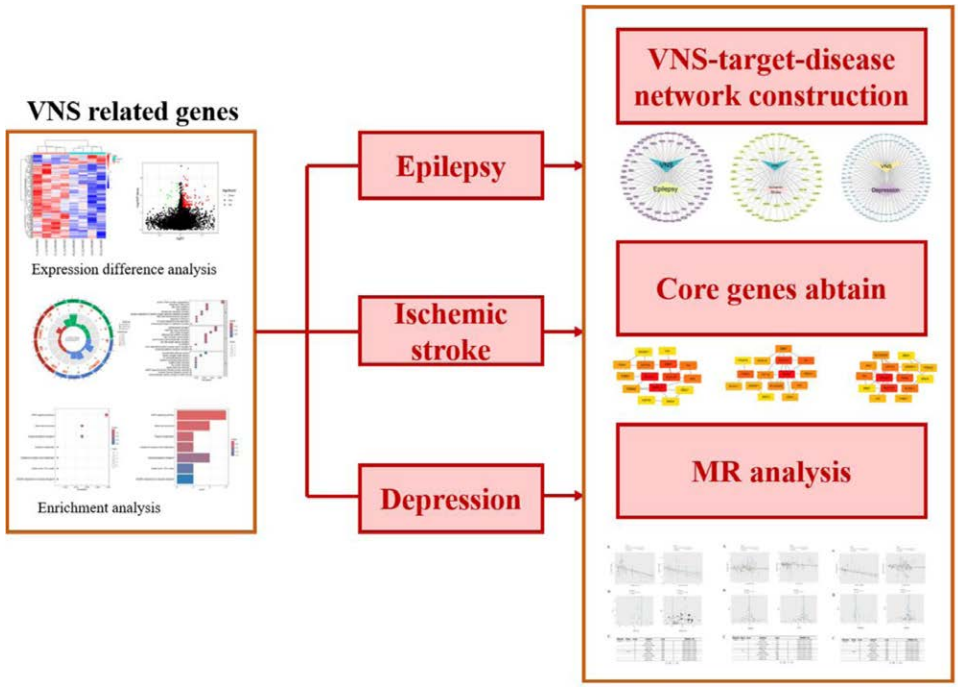


Figure 1. Flowchart showing the study design. MR = Mendelian randomization, VNS = vagus nerve stimulation.

2.4. Obtaining intersecting genes between VNS-associated targets and disease-associated targets

The "intersect" function in R software was used to perform intersection operations between the screened VNS-related targets and the targets related to epilepsy, IS, and depression, respectively. The Venn diagram was created using the "venndiagram" package in R software.

2.5. Construction of the PPI network and analysis of its topological properties

From the Search Tool for Recurring Instances of Neighbouring Genes database (STRING, website: https://cn.string-db.org/), the PPI network among target proteins was obtained with the confidence score set to be >0.4. The relevant files downloaded from the STRING database were imported into the Cytoscape 3.9.1 software (an open-source bioinformatics analysis software jointly developed by research institutions such as the Institute for Systems Biology [Leroy Hood Lab], the University of California, San Diego [Trey Ideker Lab], the University of California, San Francisco [Bruce Conklin Lab], Memorial Sloan-Kettering Cancer Center [Chris Sander Lab], and the Pasteur Institute [Benno Schwikowski Lab]). The drug–target–disease network diagram was constructed using this software. The Neighborhood Component Centrality (MNC) algorithm in the CytoHubba plugin of the Cytoscape software was employed to analyze the relationship network among the intersecting targets. MNC algorithm is a method that can evaluate the centrality of nodes within their neighborhood components. Consequently, the core nodes were calculated, and the top 15 core genes in this network were screened out.

2.6. MR analysis

To explore whether there exists a causal relationship between the screened core targets of VNS and epilepsy, IS, and depression, we took each of the screened key targets of VNS as an exposure factor, and each of the 3 diseases as an outcome variable, followed by conducting an MR analysis. We used the "TwoSampleMR" package in R software to screen for single nucleotide polymorphisms (SNPs) that are significantly associated with the exposure factors as IVs ($P < 5 \times 10^{-8}$). Subsequently, we removed the IVs with strong linkage disequilibrium ($r^2 = 0.3$, kb = 10,000). To meet the exclusion restriction assumption and reduce the bias caused by weak IVs, this study used the formula $F = (R^2 \times [N - 1 - K])/(K \times [1 - R^2])$ to screen out weak IVs with an F value <10. Multiple MR methods were employed to determine the associations between key target genes and diseases. These methods included MR Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode. Among these, the IVW method was the primary approach. If the P value obtained from the IVW method was <.05, it indicated a potential causal association. In addition, when the calculated odds ratio (OR) was >1, it meant that the exposure factor was a risk factor; an OR <1 indicated that the exposure factor was a protective factor for the outcome variable. The results were presented via scatter plots, funnel plots, and forest plots. Subsequently, the reliability of the MR results was evaluated through heterogeneity tests, horizontal pleiotropy tests, and leave-one-out analysis. In the heterogeneity test, a P value >.05 indicated the absence of heterogeneity. In the horizontal pleiotropy test, a P value >.05 represented the absence of horizontal pleiotropy.

3. Result

3.1. Identification of VNS-related genes and exploration of their potential biological functions

The VNS-related dataset GSE210043 was obtained from the Gene Expression Omnibus database. Compared with the control group, we identified 159 DEGs in the VNS treatment group (Fig. 2A and B). Gene Ontology results indicated that the DEGs were involved in biological processes such as "protein-RNA complex organization," "fatty acid oxidation," and "carbon-oxygen lyase activity" (Fig. 2C and D). Kyoto

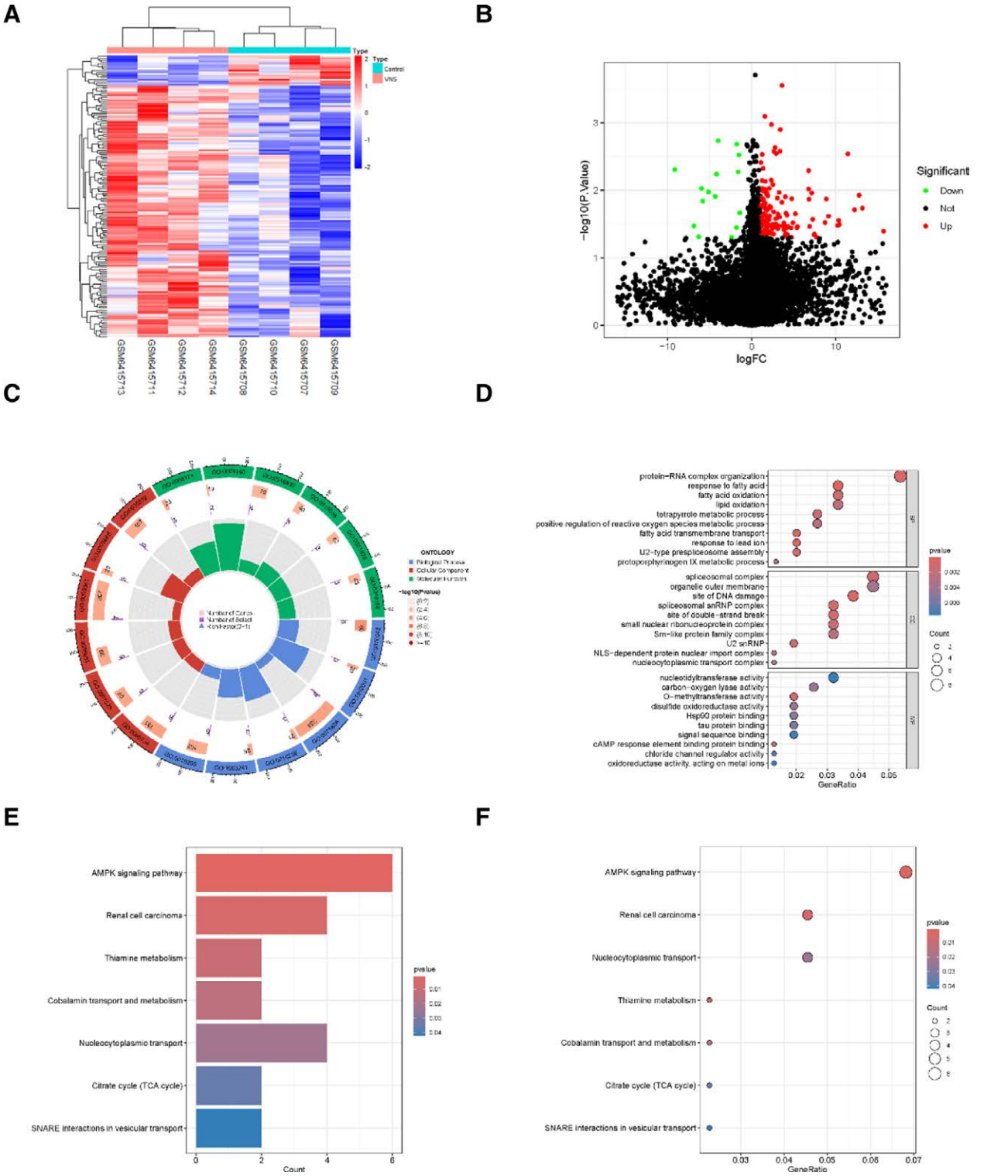


Figure 2. Screening of DEGs between normal and VNS treatment groups in the GSE210043 dataset. (A) The heatmap plot and (B) volcano for the expression patterns of the DEGs. (C) A circular graph displaying the enrichment of DEGs in different GO categories (BP, CC, MF). The outer circle shows the 6 most significant pathways and their respective numbers and significance of enriched genes and target genes, while the inner circle represents the enrichment level of the functional category or pathway. (D) Bubble plot of GO enrichment analysis. (E), (F) Bar plot and bubble plot of KEGG enrichment analysis, which show the details of the top 7 significantly enriched pathways. BP = biological process, CC = cellular component, DEGs = differentially expressed genes, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, VNS = vagus nerve stimulation.

Encyclopedia of Genes and Genomes enrichment analysis showed that these genes were enriched in metabolism-related pathways, including the "AMPK signaling pathway," "Thiamine metabolism," and "Cobalamin transport and metabolism" (Fig. 2E and F).

3.2. Collection of epilepsy-related genes and obtaining the intersection of epilepsy-related genes and VNS-related genes

We obtained 7741 epilepsy-related genes from the GeneCards database. By taking the intersection with the 159 VNS-related genes, we got 73 overlapping genes (Fig. 3A).

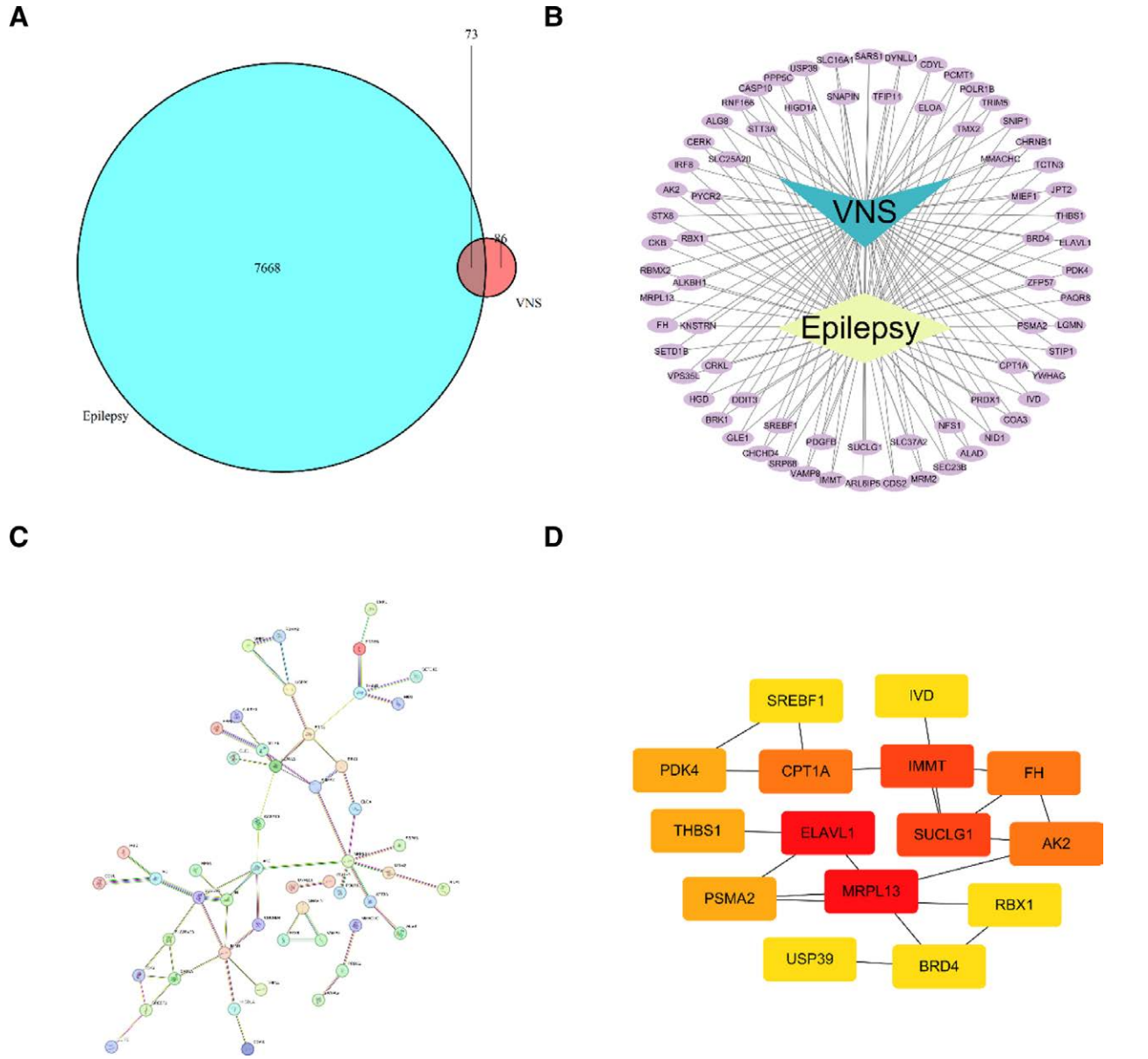


Figure 3. (A) Venn diagram of VNS and epilepsy-related targets. (B) "Drug–targets–disease" network of VNS against epilepsy. The diamond in green represents epilepsy, the "V" in blue represents VNS, and the ellipses in violet represent the intersection targets. (C) PPI network of targets generated using STRING 12.0. Nodes represent proteins. Edges represent PPIs. (D) The top 15 targets with the highest score obtained by the MNC algorithm, which were regarded as the core targets among the intersecting targets of VNS-epilepsy. MNC = Neighborhood Component Centrality, PPI = protein–protein interaction, VNS = vagus nerve stimulation.

3.3. Analysis of "VNS-target-Epilepsy" network

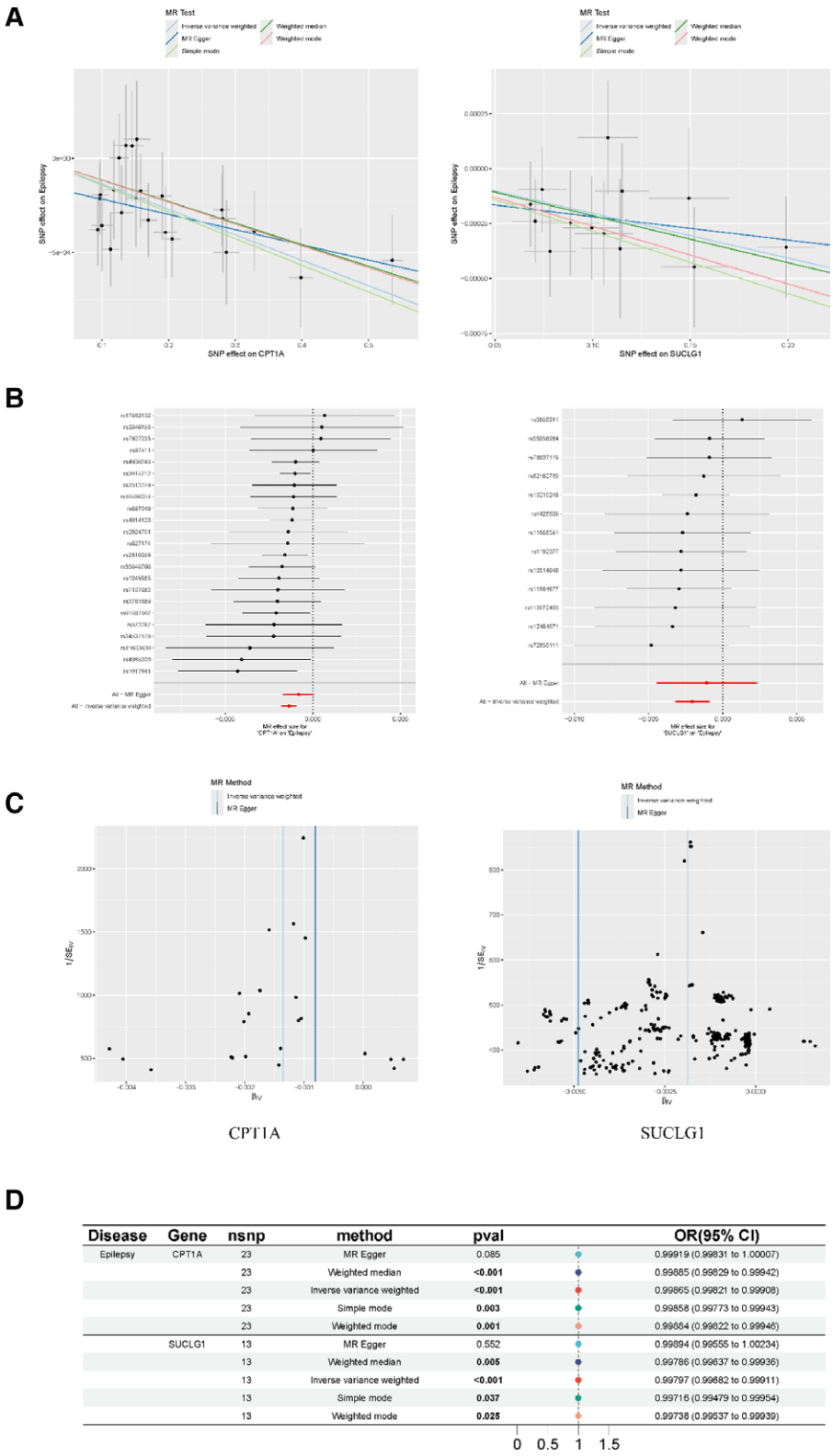
The "VNS-target-Epilepsy" network was constructed using Cytoscape_v3.9.1, which consisted of 146 edges and 75 nodes (Fig. 3B). As can be seen, VNS treats epilepsy by influencing 73 target genes.

3.4. PPI network analysis of the intersecting genes between VNS and epilepsy

The PPI network of the intersecting targets between VNS and epilepsy was obtained using the STRING database (Fig. 3C). The CytoHubba plugin in the Cytoscape v3.9.1 software was used for analysis, and the top 15 core genes in the network were obtained, including *SREBF1*, *IMMT*, *PDK4*, *PSMA2*, *RBX1*, *USP39*, *ELAVL1*, *THBS1*, *SUCLG1*, *IVD*, *CPT1A*, *FH*, *AK2*, *MRPL13*, and *BRD4* (Fig. 3D).

3.5. MR analysis of the causal relationship between core genes of VNS-epilepsy and epilepsy

The top 15 core genes among the intersecting genes of VNS-epilepsy were respectively used as exposure factor, and epilepsy was regarded as the outcome in the MR analysis (Fig. 4). The scatter plot revealed that *CPT1A* and *SUCLG1* were negatively correlated with epilepsy (Fig. 4A). In the forest plot, the MR effect size was <0, indicating that *CPT1A* and *SUCLG1* were the protective factors for epilepsy (Fig. 4B). The funnel plot of causal effects is approximately symmetrical (Fig. 4C). The results suggested that *CPT1A* and *SUCLG1* might be considered as protective factors for epilepsy. As shown in Figure 4D, the results of the "Inverse variance weighted" method (OR = 0.99865, 95% confidence interval [95% CI] = 0.99821–0.99908, $P < .001$) and the "Weighted median" method (OR = 0.99885, 95% CI = 0.99829–0.99942, $P < .001$) indicated a causal relationship between *CPT1A* and



Disease	Gene	nsnp	method	pval	OR(95% CI)
Epilepsy	CPT1A	23	MR Egger	0.085	0.99919 (0.99831 to 1.00007)
		23	Weighted median	**<0.001**	0.99885 (0.99829 to 0.99942)
		23	Inverse variance weighted	**<0.001**	0.99865 (0.99821 to 0.99908)
		23	Simple mode	**0.003**	0.99858 (0.99773 to 0.99943)
		23	Weighted mode	**0.001**	0.99884 (0.99822 to 0.99946)
	SUCLG1	13	MR Egger	0.552	0.99894 (0.99555 to 1.00234)
		13	Weighted median	**0.005**	0.99786 (0.99637 to 0.99936)
		13	Inverse variance weighted	**<0.001**	0.99797 (0.99682 to 0.99911)
		13	Simple mode	**0.037**	0.99716 (0.99479 to 0.99954)
		13	Weighted mode	**0.025**	0.99738 (0.99537 to 0.99939)

0 0.5 1 1.5

Figure 4. MR analysis. (A) Scatter plots showing the causal effects of *CPT1A* and *SUCLG1* on the risk of epilepsy. (B) Forest plots showing the causal effects of *CPT1A* and *SUCLG1* each SNP on the risk of epilepsy. (C) Funnel plots demonstrating the overall heterogeneity of MR estimates for the effects of *CPT1A* and *SUCLG1* on epilepsy. (D) The causal effect of *CPT1A* and *SUCLG1* on epilepsy. Error bars indicate the 95% CIs for the estimates. CI = confidence interval, MR = Mendelian randomization, OR = odds ratio, SNP = single nucleotide polymorphism.

epilepsy ($P < .05$), and *CPT1A* served as a protective factor for epilepsy. In addition, in the "Inverse variance weighted" analysis, *SUCLG1* was shown to have a causal relationship with epilepsy ($P < .05$) with an OR of 0.99797 (95% CI = 0.99682–0.99911, $P < .001$), indicating that it is a protective factor for epilepsy. Although the results of the other 4 methods did not reach statistical significance, they still suggested the potential protective effect of *SUCLG1* in epilepsy.

To ensure the robustness of the results, we conducted a leave-one-out analysis. Specifically, after excluding each SNP, we performed MR analysis using the remaining SNPs. The results showed that there was no significant change in the outcomes after excluding any single SNP (Fig. S1A and B, Supplemental Digital Content, https://links.lww.com/MD/R660). Therefore, based on the MR analysis, we determined that the 2 genes, *CPT1A* and *SUCLG1*, could potentially be regarded as marker genes for VNS treatment of epilepsy.

3.6. Collect genes related to IS and obtain the intersection of genes related to IS and genes related to VNS

We obtained 6134 genes related to IS from the GeneCards database. By intersecting these genes with the 159 VNS-related genes, we got 54 intersecting genes (Fig. 5A).

3.7. Construction of the "VNS-target-IS" network

The "VNS-target-IS" network was constructed using Cytoscape_v3.9.1, which consisted of 108 edges and 56 nodes (Fig. 5B). The 54 genes depicted in the figure serve as both target genes of VNS and pathogenic genes associated with IS.

3.8. PPI network analysis of the intersecting genes between VNS and IS

The interaction network of the intersecting targets of VNS and IS was obtained using the STRING database (Fig. 5C). The CytoHubba plugin in the Cytoscape_v3.9.1 software was used for analysis, and the top 15 core genes in the network were obtained, including *IMMT*, *PDGFB*, *HIGD1A*, *SUCLG1*, *FH*, *THBS1*, *CPT1A*, *ACAA2*, *PRDX1*, *ELAVL1*, *SREBF1*, *SLC25A20*, *IVD*, *DDIT3*, and *CDYL* (Fig. 5D).

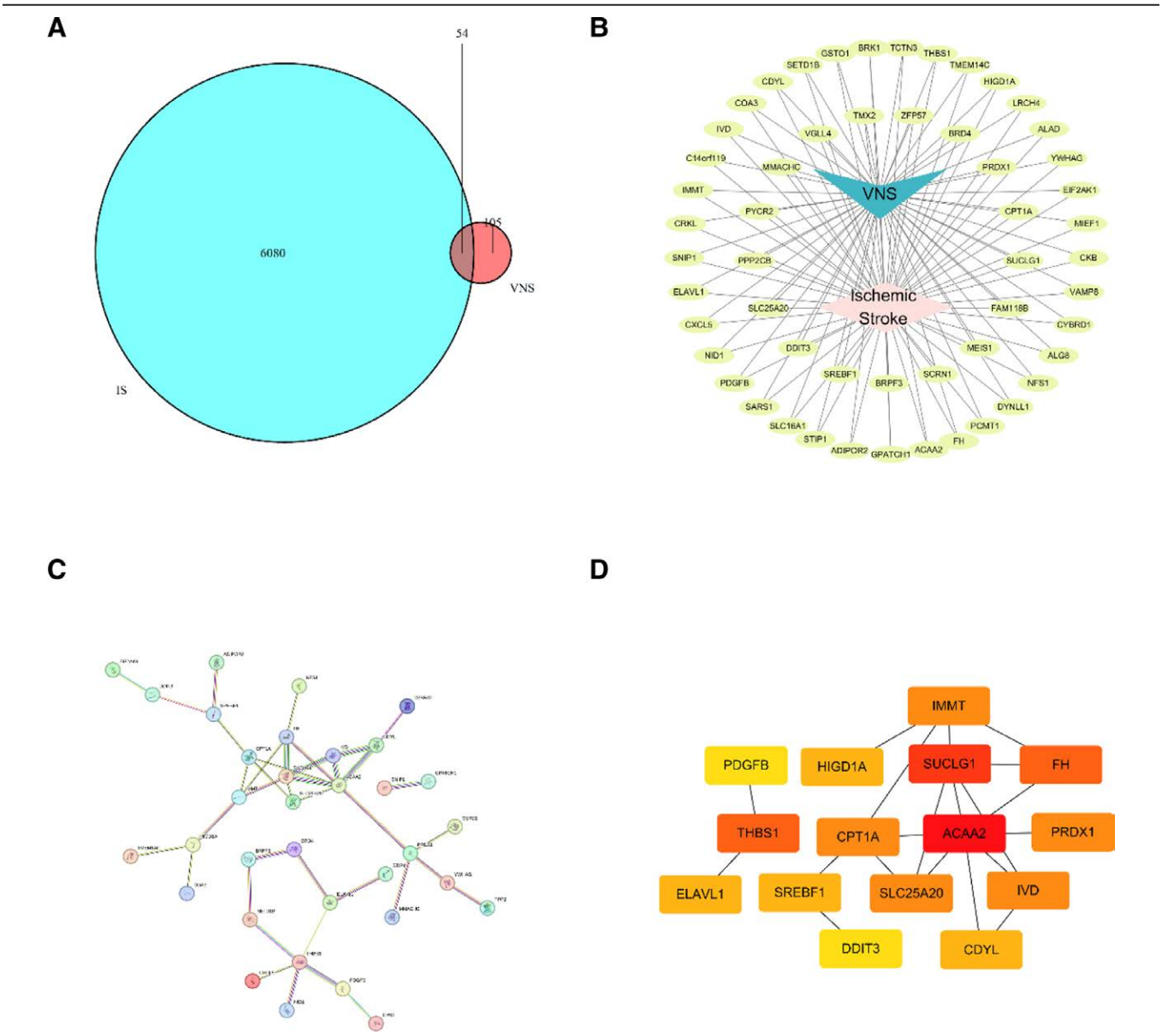


Figure 5. (A) Venn diagram of VNS and IS-related targets. (B) "Drug–targets–disease" network of VNS against IS. The diamond in pink represents ischemic stroke, the "V" in blue represents VNS, and the ellipses in green represent the intersection targets. (C) PPI network of targets generated using STRING 12.0. Nodes represent proteins. Edges represent PPIs. (D) The top 15 targets with the highest score obtained by the MNC algorithm, which were regarded as the core targets among the intersecting targets of VNS-IS. IS = ischemic stroke, MNC = Neighborhood Component Centrality, PPI = protein–protein interaction, VNS = vagus nerve stimulation.

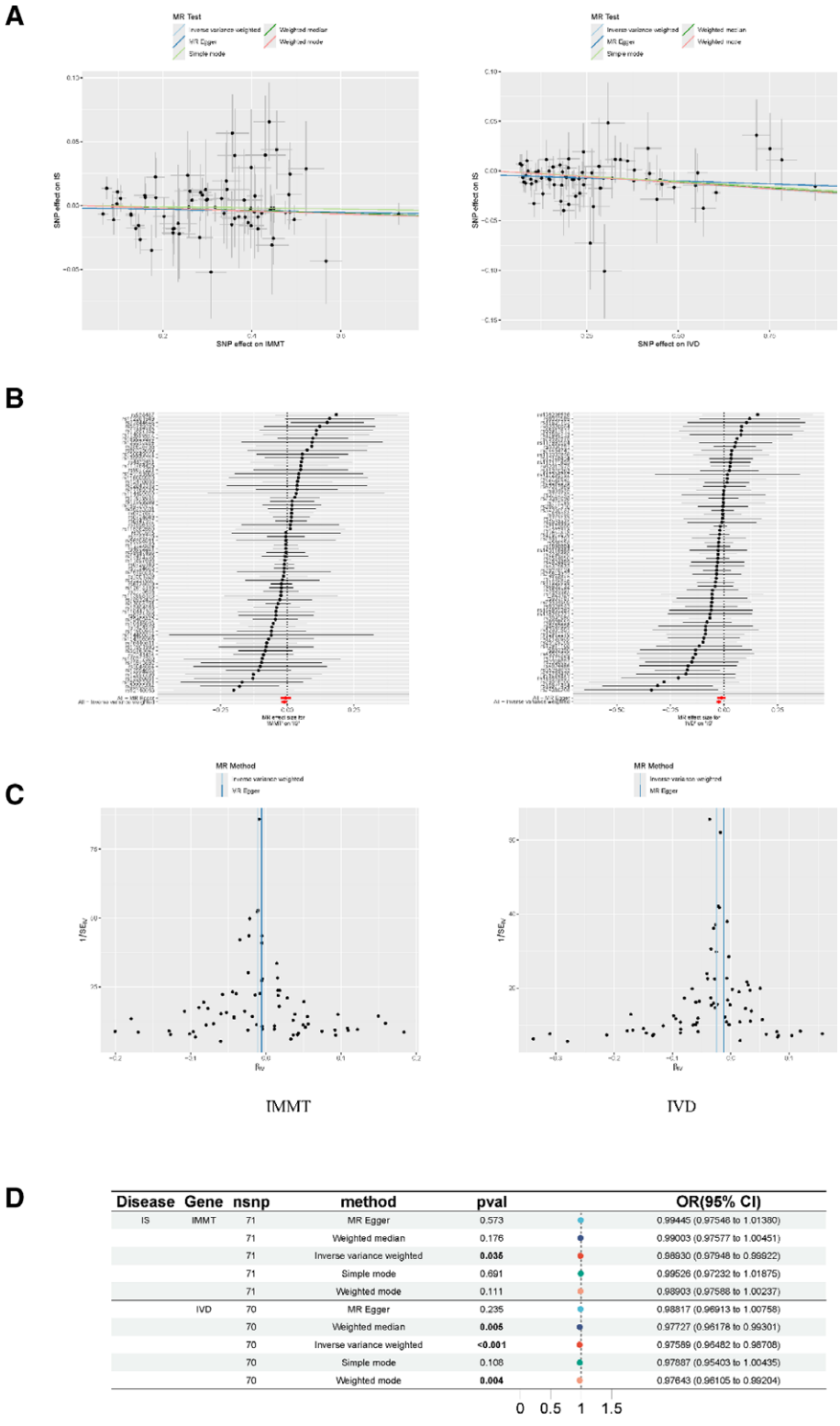


Figure 6. MR analysis. (A) Scatter plots showing the causal effects of *IMMT* and *IVD* on the risk of IS. (B) Forest plots showing the causal effects of *IMMT* and *IVD* each SNP on the risk of IS. (C) Funnel plots demonstrating the overall heterogeneity of MR estimates for the effects of *IMMT* and *IVD* on IS. (D) The causal effect of *IMMT* and *IVD* on IS. Error bars indicate the 95% CIs for the estimates. CI = confidence interval, IS = ischemic stroke, MR = Mendelian randomization, OR = odds ratio, SNP = single nucleotide polymorphism.

3.9. Results of MR analysis between core genes of VNS-IS intersection genes and IS

We selected the 15 core genes screened in Figure 5D. Sequentially taking each of them as the exposure factor and IS as the outcome variable, we carried out the MR analysis. The analysis showed that *IMMT* and *IVD* presented as protective factors for IS (Fig. 6). The negative correlation between *IMMT*, *IVD*, and IS can be observed from the scatter plot (Fig. 6A). As depicted in Figure 6B, the MR effect size registered below 0. This finding indicates that *IMMT*

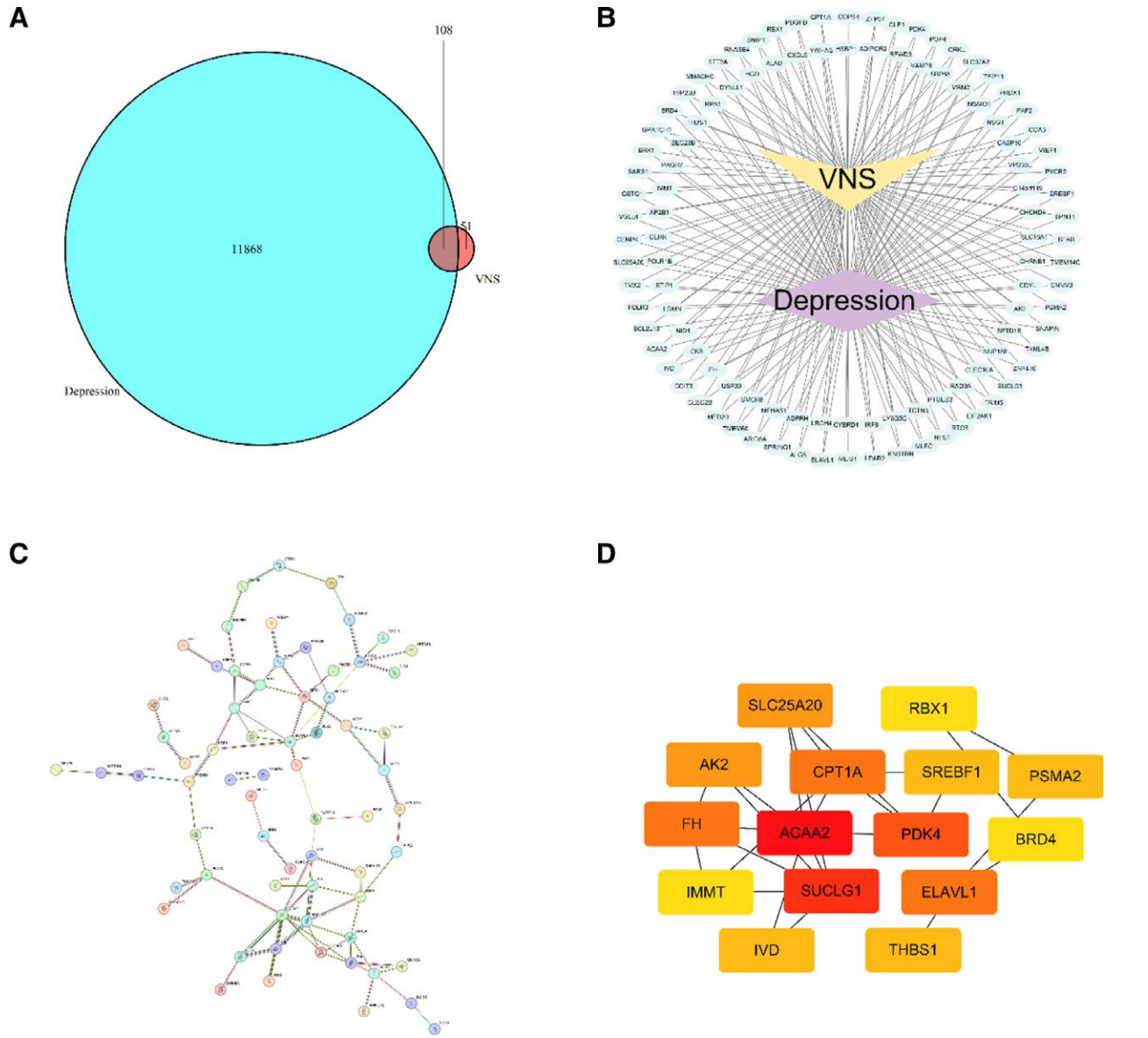


Figure 7. (A) Venn diagram of VNS and depression-related targets. (B) "Drug–targets–disease" network of VNS against depression. The diamond in violet represents depression, the "V" in yellow represents VNS, and the ellipses in blue represent the intersection targets. (C) PPI network of targets generated using STRING 12.0. Nodes represent proteins. Edges represent PPIs. (D) The top 15 targets with the highest score obtained by the MNC algorithm, which were regarded as the core targets among the intersecting targets of VNS-depression. MNC = Neighborhood Component Centrality, PPI = protein–protein interaction, VNS = vagus nerve stimulation.

and *IVD* act as protective factors against IS. The funnel plot of causal effects is approximately symmetrical (Fig. 6C).

In the analysis of *IMMT*, 71 SNPs were employed. The "Inverse variance weighted" analysis yielded an OR of 0.98930, with a 95% CI ranging from 0.97948 to 0.99922 ($P < .05$; Fig. 6D). These results suggest that *IMMT* acts as a protective factor against IS.

For the analysis of *IVD*, 70 SNPs were used. As shown in Figure 6D, the "Inverse variance weighted" method (OR = 0.97589, 95% CI = 0.96482–0.98708, $P < .001$), "Weighted median" method (OR = 0.97727, 95% CI = 0.96178–0.99301, $P = .005$), and "Weighted mode" method (OR = 0.97643, 95% CI = 0.96105–0.99204, $P = .004$) all indicated that *IVD* is a protective factor for IS.

The results of the leave-one-out analysis showed that excluding any single SNP did not significantly affect the results (Fig. S1C and D, Supplemental Digital Content, https://links.lww.com/MD/R660).

3.10. Collect genes related to depression and obtain the intersection of genes related to depression and genes related to VNS

We retrieved 11,976 genes related to depression from the GeneCards database. Subsequently, we calculated the intersection of these genes with the 159 genes related to VNS. Eventually, 108 intersecting genes were obtained, as specifically depicted in Figure 7A.

3.11. Construction of the "VNS-target-depression" network

The "VNS-target-Depression" network was constructed using the Cytoscape_v3.9.1 software. In the structure of this network, 216 edges and 110 nodes were included (Fig. 7B). The figure shows that VNS treats depression by affecting 108 target genes.

3.12. PPI network analysis of the intersecting genes between VNS and depression

By leveraging the STRING database, the interaction network of the intersection targets between VNS and depression was acquired successfully (Fig. 7C). Subsequently, this network was analyzed using the CytoHubba plugin within the Cytoscape_v3.9.1 software, enabling the determination of the top 15 core genes in the network. These core genes are *SLC25A20*, *RBX1*, *AK2*, *CPT1A*, *SREBF1*, *PSMA2*, *FH*, *ACAA2*, *PDK4*, *BRD4*, *IMMT*, *SUCLG1*, *ELAVL1*, *IVD*, and *THBS1*, as shown in Figure 7D.

3.13. Results of MR analysis between core genes of VNS-depression intersection genes and depression

In this research, we initiated our study from the 15 core genes selected in Figure 7D. Each of these genes was separately considered as an exposure factor, with depression designated as the outcome variable, to conduct MR analysis (Fig. 8). The scatter plot in Figure 8A demonstrated an inverse correlation between *PSMA2*, *IMMT*, and depression. In the forest plot, given that the MR effect size was below zero, it suggested that *PSMA2* and *IMMT* served as protective factors for depression (Fig. 8B). As shown in Figure 8C, the funnel plot depicting causal effects exhibits approximate symmetry.

The final results are presented in Figure 8D. For the analysis of *IMMT*, 84 SNPs were used. The "Inverse variance weighted" method (OR = 0.98241, 95% CI = 0.97411–0.99077, $P < .001$), the "Weighted median" method (OR = 0.98372, 95% CI = 0.97258–0.99499, $P = .005$), and the "Weighted mode" method (OR = 0.97896, 95% CI = 0.96803–0.99001, $P < .001$), all indicated that *IMMT* is a protective factor for depression.

When analyzing *PSMA2*, 26 SNPs were utilized. Four methods, specifically the "Inverse variance weighted" method (OR = 0.95310, 95% CI = 0.93188–0.97481, $P < .001$), the "Weighted median" method (OR = 0.93843, 95% CI = 0.90815–0.96972, $P < .001$), the "Simple mode" method (OR = 0.93245, 95% CI = 0.89034–0.97654, $P = .007$), and the "Weighted mode" method (OR = 0.93245, 95% CI = 0.89894–0.96721, $P < .001$), all demonstrated that *PSMA2* is a protective factor for depression (Fig. 8D).

The leave-one-out analysis further validated the reliability of the results (Fig. S1E and F, Supplemental Digital Content, https://links.lww.com/MD/R660).

4. Discussion

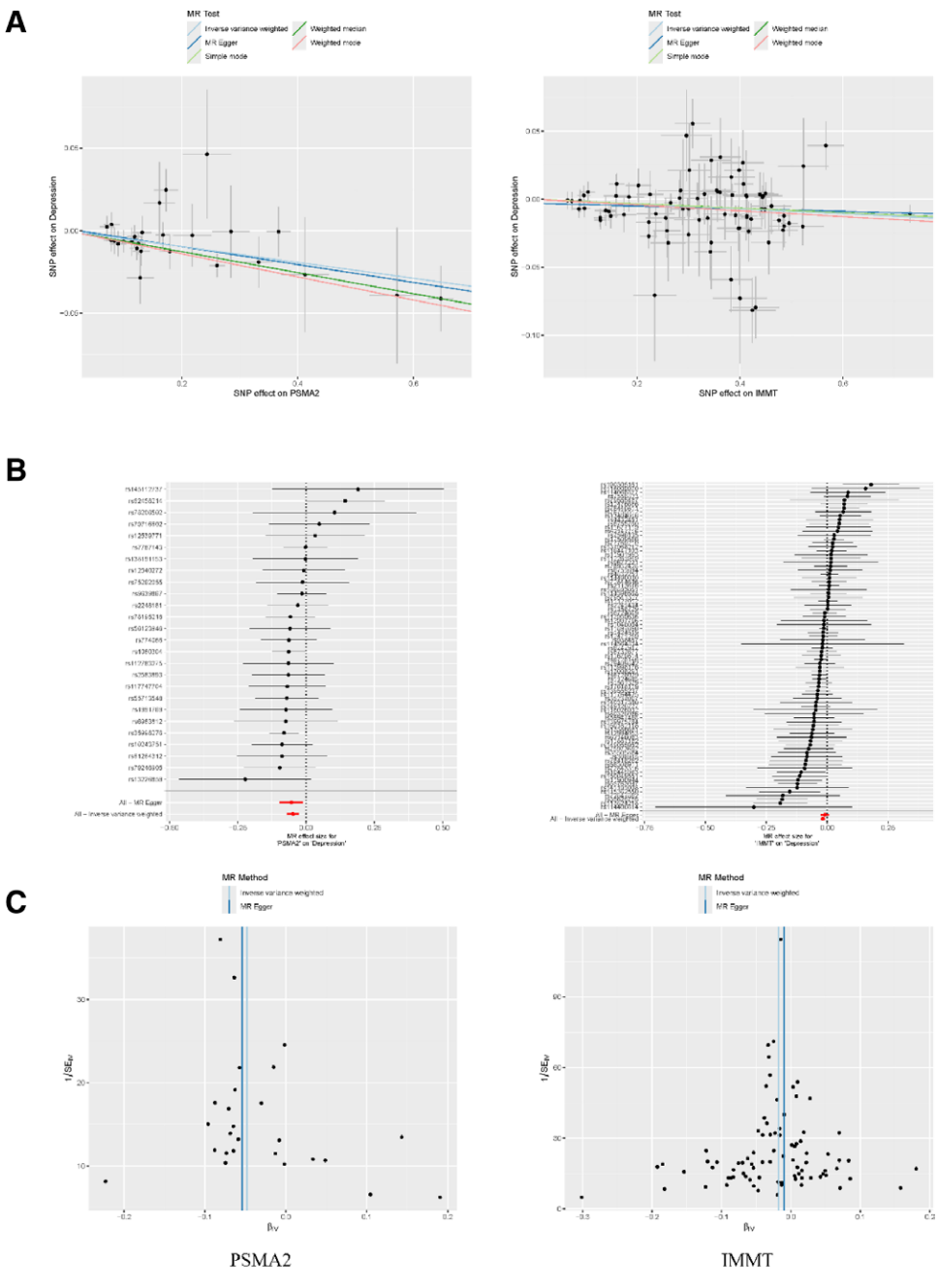
In this study, network pharmacology, bioinformatics, and MR methods were comprehensively applied to deeply explore the relationships between the core target genes of VNS and epilepsy, IS, and depression.

By analyzing the differential gene expression between the VNS treatment group and the control group, we identified 159 DEGs. These genes are involved in biological processes such as "protein-RNA complex organization," "fatty acid oxidation," and "carbon-oxygen lyase activity," and are enriched in metabolism-related pathways such as the "AMPK signaling pathway", "Thiamine metabolism," and "Cobalamin transport and metabolism."

Further analysis revealed that there were 73 overlapping genes between VNS-related genes and epilepsy-related genes. The constructed "VNS-target-epilepsy" network and PPI network screened out 15 core genes. MR analysis indicated that *CPT1A* and *SUCLG1* might be protective factors and potential marker genes for VNS in the treatment of epilepsy. Carnitine Palmitoyl-Transferase1A (*CPT1A*) is the rate-limiting enzyme in the process of fatty acid β-oxidation.[25] Defects in its function or abnormal regulation can lead to metabolic disorders such as type 2 diabetes and obesity,[26] and it could also lead to various cancers.[26,27] Lipids account for approximately 50% of the dry weight of the brain, making the brain the organ with the highest lipid content after adipose tissue. Lipids and their metabolic intermediates are crucial for the structure and function of the brain. They function as energy substrates, structural components of cell membranes, and bioactive molecules.[28] Imbalance in lipid metabolism is a key pathological mechanism underlying the occurrence of epilepsy, and it may act on epilepsy through pathways such as cellular ferroptosis, lipophagy, and immune regulation of gut microbiota.[29] This finding suggests that VNS may influence the occurrence and development of epilepsy by regulating *CPT1A*, thereby modulating the fatty acid β-oxidation process and subsequently regulating energy metabolism. *SUCLG1* is involved in the tricarboxylic acid cycle and is crucial for maintaining cellular energy homeostasis.[30,31] The brain consumes approximately 20% of the energy supplied by glucose and is the primary organ that consumes glucose. Previous reports have proposed that dysregulation of glucose metabolism associated with neuroinflammation may play a crucial role in the pathogenesis of epilepsy. It mainly involves the metabolic reprogramming of microglia, which limits the amount of glucose available to neurons, impairs adenosine triphosphate-dependent physiological processes, and consequently leads to increased neuronal excitability.[28] Therefore, VNS may treat epilepsy by regulating glucose metabolism through *SUCLG1*. Its protective role in epilepsy once again suggests that VNS may exert its antiepileptic effect by regulating energy metabolism-related pathways.

In the analysis of the relationship between VNS and IS, there were 54 overlapping genes between the IS-related genes obtained from the GeneCards database and the VNS-related genes. The constructed "VNS-target-IS" network and PPI network screened out 15 core genes. MR analysis revealed that *IMMT* and *IVD* are protective factors for IS. *IMMT*, also known as Mic60, is an inner mitochondrial membrane protein. It is involved in the assembly of mitochondrial respiratory chain complexes and is crucial for maintaining normal mitochondrial functions.[32–34] In stroke patients, disordered energy metabolism and the subsequent inflammation triggered by oxidative stress pose significant obstacles to functional recovery. During cerebral ischemia and reperfusion injury, the proper distribution and maintenance of mitochondrial function are crucial for maintaining energy homeostasis and regulating the inflammatory response. Therefore, VNS may protect patients with IS by maintaining normal mitochondrial function through *IMMT*.[35] *IVD* is involved in the catabolism of branched-chain amino acids.[36] Shen et al have shown that in IS, the accumulation of branched-chain amino acids can exacerbate microglia-induced neuroinflammation by activating the AKT/STAT3/NF-κB signaling pathway.[37] In addition, previous studies have suggested that there are significant differences in amino acid metabolism between patients in the convalescent phase of IS and healthy individuals.[38] Therefore, we speculate that VNS may protect patients with IS by maintaining normal amino acid metabolism through *IVD*. Its protective effect in IS may be related to the regulation of energy metabolism and intracellular homeostasis. The above results suggest that VNS may exert a protective effect on IS by regulating mitochondrial function and pathways related to amino acid metabolism.

In the research on depression, there were 108 overlapping genes between the VNS-related genes and the depression-related genes. The constructed "VNS-target-depression" network and PPI network screened out 15 core genes. MR analysis indicates that *PSMA2* and *IMMT* are protective factors against depression. *PSMA2* is a component of the proteasome and is involved in the protein degradation process.[39,40] Ubiquitin marks misfolded or damaged proteins during the ubiquitination process. This process



Disease	Gene	nsnp	method	pval	OR(95% CI)
Depression	IMMT	84	MR Egger	0.260	0.99031 (0.97381 to 1.00710)
		84	Weighted median	**0.005**	0.98372 (0.97258 to 0.99499)
		84	Inverse variance weighted	**<0.001**	0.98241 (0.97411 to 0.99077)
		84	Simple mode	0.101	0.98355 (0.96448 to 1.00299)
		84	Weighted mode	**<0.001**	0.97896 (0.96803 to 0.99001)
	PSMA2	26	MR Egger	**0.023**	0.94737 (0.90698 to 0.98956)
		26	Weighted median	**<0.001**	0.93843 (0.90815 to 0.96972)
		26	Inverse variance weighted	**<0.001**	0.95310 (0.93188 to 0.97481)
		26	Simple mode	**0.007**	0.93245 (0.89034 to 0.97654)
		26	Weighted mode	**<0.001**	0.93245 (0.89694 to 0.96721)

0 0.5 1 1.5

Figure 8. MR analysis. (A) Scatter plots showing the causal effects of *IMMT* and *PSMA2* on the risk of depression. (B) Forest plots showing the causal effects of *IMMT* and *PSMA2* each SNP on the risk of depression. (C) Funnel plots demonstrating the overall heterogeneity of MR estimates for the effects of *IMMT* and *PSMA2* on depression. (D) The causal effect of *IMMT* and *PSMA2* on depression. Error bars indicate the 95% CIs for the estimates. CI = confidence interval, MR = Mendelian randomization, OR = odds ratio, SNP = single nucleotide polymorphism.

is carried out by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). Proteins marked by ubiquitination will be degraded by the proteasome. The ubiquitin-proteasome system is involved in development, neuronal function, and cell signaling during stress, inflammation, and apoptosis.[41] Proteasome dysfunction may be associated with

insufficient clearance of misfolded proteins in the cytoplasm, nucleus, and endoplasmic reticulum. This can subsequently lead to intracellular protein aggregation, cytotoxicity, and cell death. Such dysfunction is related to various neurological diseases.[42] Therefore, VNS may treat depression by regulating *PSMA2* to maintain intracellular protein homeostasis. As mentioned previously, *IMMT* is an inner mitochondrial membrane protein. Mitochondria play a crucial role in the pathogenesis of depression.[43] Impaired mitochondrial energy metabolism and damaged quality control systems have been proven to be associated with the development of depression.[44] Moreover, some antidepressants exert their effects by improving mitochondrial function.[44,45] Therefore, VNS may exert its antidepressant effect by regulating *IMMT* to maintain the normal function of mitochondria.

However, this study also has certain limitations. First, although we have screened out potential core target genes through various bioinformatics methods and database analyses, these results still need to be further verified experimentally. This is to clarify the specific mechanisms of action of these genes in the process of VNS treating diseases. Second, due to the limitations of the database, the GSE210043 dataset used in this study only includes peripheral blood samples from 4 patients in the VNS treatment group and 4 patients in the control group. The small sample size may reduce the statistical power to detect true DEGs, increasing the risk of false-positive or false-negative results. In the future, cohort studies with larger sample sizes are needed to validate these findings. In addition, this study mainly focused on genetic-level analysis. However, the occurrence and development of neurological diseases is a complex multifactor process, involving the interaction between genes and environmental factors. In subsequent studies, more environmental factors could be considered for comprehensive analysis.

In summary, this study has revealed the potential core target genes and underlying molecular mechanisms possibly involved in VNS treatment of epilepsy, IS, and depression through the integration of multiple methods. Although there are limitations, these findings provide important clues for further in-depth research on the mechanisms of VNS in treating neurological diseases, and hold promise for the development of more precise and effective treatment strategies.

5. Conclusion

This study comprehensively and in-depth explored the relationships between the core target genes of VNS and 3 diseases (epilepsy, IS, and depression) by integrating network pharmacology, bioinformatics, and MR. The study found that VNS-related genes were involved in a variety of important biological processes and metabolic-related pathways. In the correlation analysis with the 3 diseases, core genes related to epilepsy, IS, and depression were respectively screened out. Moreover, through MR analysis, it was determined that some core genes have a protective effect on the corresponding diseases. Specifically, *CPT1A* and *SUCLG1* may be protective factors and potential marker genes for VNS treatment of epilepsy; *IMMT* and *IVD* have a protective effect on IS; *PSMA2* and *IMMT* are protective factors for VNS treatment of depression. These results uncover the potential molecular mechanisms possibly involved in the treatment of these 3 neurological diseases by VNS, providing new perspectives and theoretical bases for a deeper understanding of the therapeutic effects of VNS.

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